



IMPERIAL AGRICULTURAL  
RESEARCH INSTITUTE, NEW DELHI.

MGIPC—S4—III-1-93—22-8-45—5,000.





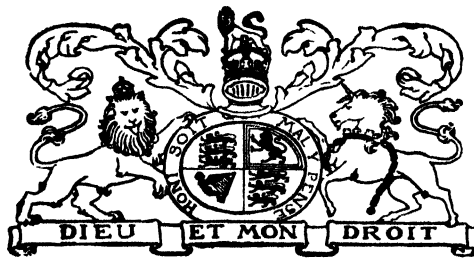






**JOURNAL OF THE MALARIA  
INSTITUTE OF INDIA.**





*All rights reserved*



# JOURNAL OF THE MALARIA INSTITUTE OF INDIA.

PUBLISHED UNDER THE AUTHORITY OF  
THE INDIAN RESEARCH FUND ASSOCIATION.

EDITOR :—BRIGADIER G. COVELL, M.D., D.P.H., D.T.M. & H., I.M.S.,  
*Director, Malaria Institute of India.*

ASSISTANT EDITOR :—MAJOR JASWANT SINGH, M.B., Ch.B., D.P.H.,  
D.T.M. & H., I.M.S.,  
*Assistant Director, Malaria Institute of India.*

Vol. IV, 1941-42.

PUBLISHED FOR  
THE INDIAN RESEARCH FUND ASSOCIATION

BY  
THACKER'S PRESS & DIRECTORIES, LTD., CALCUTTA

*Price per Volume : Seven Rupees Eight Annas (or Eleven Shillings Three Pence),  
including Postage.*

*Single Copies : Three Rupees (or Four Shillings Six Pence), including Postage.*



Printed by J. F. PARR at  
THACKER'S PRESS & DIRECTORIES, LTD.,  
6, Bentinck Street, CALCUTTA.



# TABLE OF CONTENTS.

Vol. IV, 1941-42.

No. 1 (June 1941).

	PAGES
COVELL, G. Antimalaria Operations in Delhi. Part III .. ..	1-13
RUSSELL, PAUL F., MULLIGAN, H. W., and MOHAN, BADRI NATH. Specific Agglutinogenic Properties of Inactivated Sporozoites of <i>P. gallinaceum</i> .. .. .	15-24
MULLIGAN, H. W., RUSSELL, PAUL F., and MOHAN, BADRI NATH. Active Immunization of Fowls against <i>Plasmodium gallinaceum</i> by Injections of Killed Homologous Sporozoites .. ..	25-34
VISWANATHAN, D. K. Experimental Malaria Control in a Hyperendemic Tea Garden in Upper Assam by the Use of Pyrocyde 20 as an Insecticidal Spray .. .. .	35-55
SENIOR WHITE, R. Observations on the Adult Habits of <i>Anopheles</i> <i>fluviatilis</i> and <i>Anopheles varuna</i> .. .. .	57-62
THOMSON, R. C. MUIRHEAD. Studies on the Behaviour of <i>Anopheles</i> <i>minimus</i> . Part IV. The Composition of the Water and the Influence of Organic Pollution and Silt .. .. .	63-102
CLARK, R. H. P., and CHOUDHURY, M. A. Observations on <i>Anopheles</i> <i>leucosphyrus</i> in the Digboi Area, Upper Assam .. ..	103-107
CHOPRA, R. N., ROY, D. N., and GHOSH, S. M. The Insecticidal and Larvicidal Action of the Essential Oils of <i>Ocimum basilicum</i> (Linn.) and <i>Ocimum sanctum</i> (Linn.) .. .. .	109-112
SEN, P. Aquatic Plants in the Ecology of Anopheline Mosquitoes ..	113-137
VISWANATHAN, D. K. The Utility of Malaria Parasite Indices in Infants in the Study of Malaria .. .. .	139-152
JAMES, E. R. Abstract. The Investigation of the High Incidence of Malaria in Mawchi Mines Area, situated in Karenni States, Burma, and the Measures adopted to Combat and Control the Disease .. .. .	153-154



## No. 2 (December 1941).

SIVALINGAM, V., and RUSTOMJEE, K. J. Spleen and Parasite Surveys in Ceylon .. .. .	155—173
SHORTT, H. E., MENON, K. P., and SEETHARAMA IYER, P. V. The Natural Host of <i>Plasmodium gallinaceum</i> (Brumpt, 1935) ..	175—178
SEETHARAMA IYER, P. V., SHORTT, H. E., and MENON, K. P. The Stage of <i>Plasmodium gallinaceum</i> found in the Incubation Period—Second Observation .. .. .	179—180
RUSSELL, PAUL F., and KNIPE, FRED W. Malaria Control by Spraying Adult Mosquitoes. Third Season's Results .. .. .	181—197
DIKSHIT, B. B. Malaria Immunity in the Rhesus Monkey ..	199—206
MENON, M. A. U. On the Abdominal Tergites of Adult <i>Mansonioides Theobaldi</i> , 1907. (Diptera, Culicidae) .. .. .	207—210
JOB, T. J. On the Comparative Efficacy and Relative Costs of Biological and Chemical Methods of Mosquito Control in Clean-weeded Railway Borrowpits at Fuleshwar, Bengal .. .. .	211—215
THOMSON, R. C. MUIRHEAD. Studies on the Behaviour of <i>Anopheles minimus</i> . Part V. The Behaviour of Adults in Relation to Feeding and Resting in Houses .. .. .	217—245
THOMSON, R. C. MUIRHEAD. Studies on the Behaviour of <i>Anopheles minimus</i> . Part VI. Observations on the Cold Weather Breeding Places .. .. .	247—255
SEN, P. On the Microsporidia infesting some Anophelines of India ..	257—261
RUSSELL, PAUL F., and RAMACHANDRA RAO, T. On Seasonal Prevalence of <i>Anopheles</i> Species in South-eastern Madras .. .. .	263—296
VISWANATHAN, D. K., DAS, SRIBAS, and OOMMEN, A. V. Malaria-carrying Anophelines in Assam, with Special Reference to the Results of Twelve Months' Dissections .. .. .	297—306
RAO, G. R. Abstract. Malaria in the Jharia Mining Settlement, Bihar .. .. .	307—309

## No. 3 (June 1942).

RUSSELL, PAUL F., MULLIGAN, H. W., and MOHAN, BADRI NATH. Active Immunization of Fowls against Sporozoites but not Trophozoites of <i>Plasmodium gallinaceum</i> by Injections of Homologous Sporozoites ..	311—319
RUSSELL, PAUL F., KNIPE, FRED W., and RAO, H. RAMANATHA. On the Intermittent Irrigation of Ricefields to Control Malaria in South India .. .. .	321—340



# Table of Contents.

vii

## PAGES

RAO, R. BHASKER, and RAMOO, H. Some Notes on the Practical Aspects of Mosquito Control in Wells and Tanks by the Use of Larvivoracious Fish .. .. .	341—347
RUSSELL, PAUL F., and JACOB, V. P. On the Epidemiology of Malaria in the Nilgiris District, Madras Presidency .. .. .	349—392
VISWANATHAN, D. K. Malaria Control by Spray-killing Adult Anophelines, Second Season's Results: With Special Reference to the Effects of this Measure on the Longevity and Infectivity of <i>Anopheles minimus</i> .. .. .	393—403
RAO, V. VENKAT. ROY, B. B., and RAO, P. JAGANNADHA. Observations on the Swarming and Pairing of <i>A. sundaiensis</i> (Rodenwaldt) and <i>A. subpictus</i> (Grassi) .. .. .	405—408
RAO, R. BHASKER, and RAMOO, H. The Control of Mosquito-Breeding in Canal Distributaries by growing Certain Plants on their Banks .. .. .	409—415
ROY, D. N., and BISWAS, T. C. On the Importance of <i>Anopheles pallidus</i> as a Carrier of Malaria in Udaipur State, Central Provinces .. .. .	417—420
KHAN, BHUPENDRA MOHAN. Abstract. Malaria in the Tista Valley, Darjeeling District .. .. .	421
PANIGRAHI, R. G. Abstract. Malaria in Puri .. .. .	423—428

## No. 4 (December 1942).

SALITERNIK, Z., and THEODOR, O. On a New Variety of <i>Anopheles turkhudi</i> from Palestine .. .. .	429—434
IYENGAR, M. O. T. Studies on Malaria in the Deltaic Region of Bengal .. .. .	435—446
DE BURCA, B., and MOHD. YUSAF. A New Variation of <i>Anopheles gambiae</i> .. .. .	447—449
WIJESUNDARA, D. P. Notes on the Mosquito Fauna of Rot-holes in Trees and Bamboo Stumps in Ceylon .. .. .	451—456
COVELL, G., and PRITAM SINGH. Malaria in the Coastal Belt of Orissa .. .. .	457—593
THOMSON, R. C. MUIRHEAD. Studies on the Behaviour of <i>Anopheles minimus</i> . Part VII. Further Studies on the Composition of the Water in Breeding Places and the Influence of Organic Pollution .. .. .	595—610
THOMSON, R. C. MUIRHEAD. Studies on the Behaviour of <i>Anopheles minimus</i> . Part VIII. The Naturalistic Control of <i>A. minimus</i> in Shallow Earth Wells .. .. .	611—614



	PAGES
KNIFE, F. W., and RUSSELL, P. F. A Demonstration Project in the Control of Rural Irrigation Malaria by Antilarval Measures ..	615—631
RAO, R. B., and RAMOO, H. Abstract. Observations on the Relative Utility of <i>Gambusia affinis</i> and <i>Panchar parvus</i> in the Control of Mosquito Breeding in Wells and Tanks .. ..	633—634
EDITORIAL NOTE. Status of <i>Anopheles culicifacies</i> as a Vector of Malaria in the Eastern Satpura Ranges .. ..	635
INDEX OF AUTHORS .. ..	637—640
INDEX OF SUBJECTS .. ..	641—642



**ERRATA.**

*Journal of the Malaria Institute of India*, Vol. III, Nos. 2 and 3, September 1940.

Page 203, line 21: Blacklock and Gordon (1925a) *should read* Blacklock and Gordon (1925a; 1925b).

Page 237, line 26: epidemic *should read* endemic.

Page 240, line 20: Sinton (*loc. cit.*) *should read* Sinton and Harbhagwan (1935).







## ANTIMALARIA OPERATIONS IN DELHI.

### Part III.

BY

LIEUT.-COLONEL G. COVELL, M.D., D.P.H., I.M.S.  
(*Director, Malaria Institute of India*).

[February 1, 1941.]

IN Part I of this series of papers, an account was given of the history of malaria in Delhi, and of the conditions existing in the urban area prior to the commencement of the present antimalaria campaign in 1936 (Covell, 1939). In Part II, the organization and methods of application of the various recurring measures employed in the campaign were described in detail (Covell and Afridi, 1939). The present article deals with the various permanent antimalarial engineering works, all of which have now been completed with the exception of one major project, the Western Jumna Canal closure scheme.

In planning the programme of antimalaria works, schemes dealing with areas where satisfactory control was impossible without the execution of permanent works, were given priority. The remaining cases were subjected to a rigid scrutiny, to determine whether it would be more economical to deal with them by permanent works, or to control them indefinitely by recurring measures. Projects falling in the latter category were deleted from the programme.

#### A. EASTERN AREA. .

The works described in this section were designed to deal with conditions arising primarily from the annual monsoon rise in the level of the River Jumna. Chief among them are a series of projects concerned directly with the Bela, the extensive low-lying tract intersected by numerous channels representing former branches of the river and covered with reeds and grasses, which is subject to flooding each year from July to September. In several cases, it has been found possible to formulate final proposals for permanent works in this tract only after three years' intensive study of the behaviour of the flood water. The various projects are here discussed in order from north to south.

(1) *Timarpur Sullage Farm scheme*.—This project was planned to control mosquito breeding in an extensive low-lying area situated to the north-east of the sullage farm, and immediately south of the terminal section of the Najafgarh Cut. In addition to local rainwater, this area, which lies adjacent to the



Timarpur clerks' colony, received the run-off from the sullage farm, and was subject to flooding from the river whenever this rose to a height sufficient to head up the water in the Najafgarh Cut. The project consisted of three parts : (i) the construction of a by-pass for the sullage water, so that this could be diverted into the Cut during the monsoon period; (ii) levelling and dressing the whole area so that it would drain into the Cut through a culvert passing beneath a raised roadway running along its northern boundary; and (iii) the provision of sluice-gates in the culvert, which can be closed to prevent water being headed back into it when the river rises (Map 1 and Plate I).

(2) *Metcalf House scheme*.—This was a comparatively small work, but the area dealt with was formerly a prolific source of mosquito breeding. It included filling and levelling operations and the provision of a small sluice-gate in the river bund.

(3) *Bela Swamp scheme*.—A depressed area immediately south of Metcalfe Nala outfall was filled and sloped so that, though it is still subject to flooding for short periods when the river level is at its highest, water no longer remains in it when the flood recedes (Plate II). To fill this area to a level exceeding that of the highest possible flood would have involved a heavy expenditure, which was not considered justifiable.

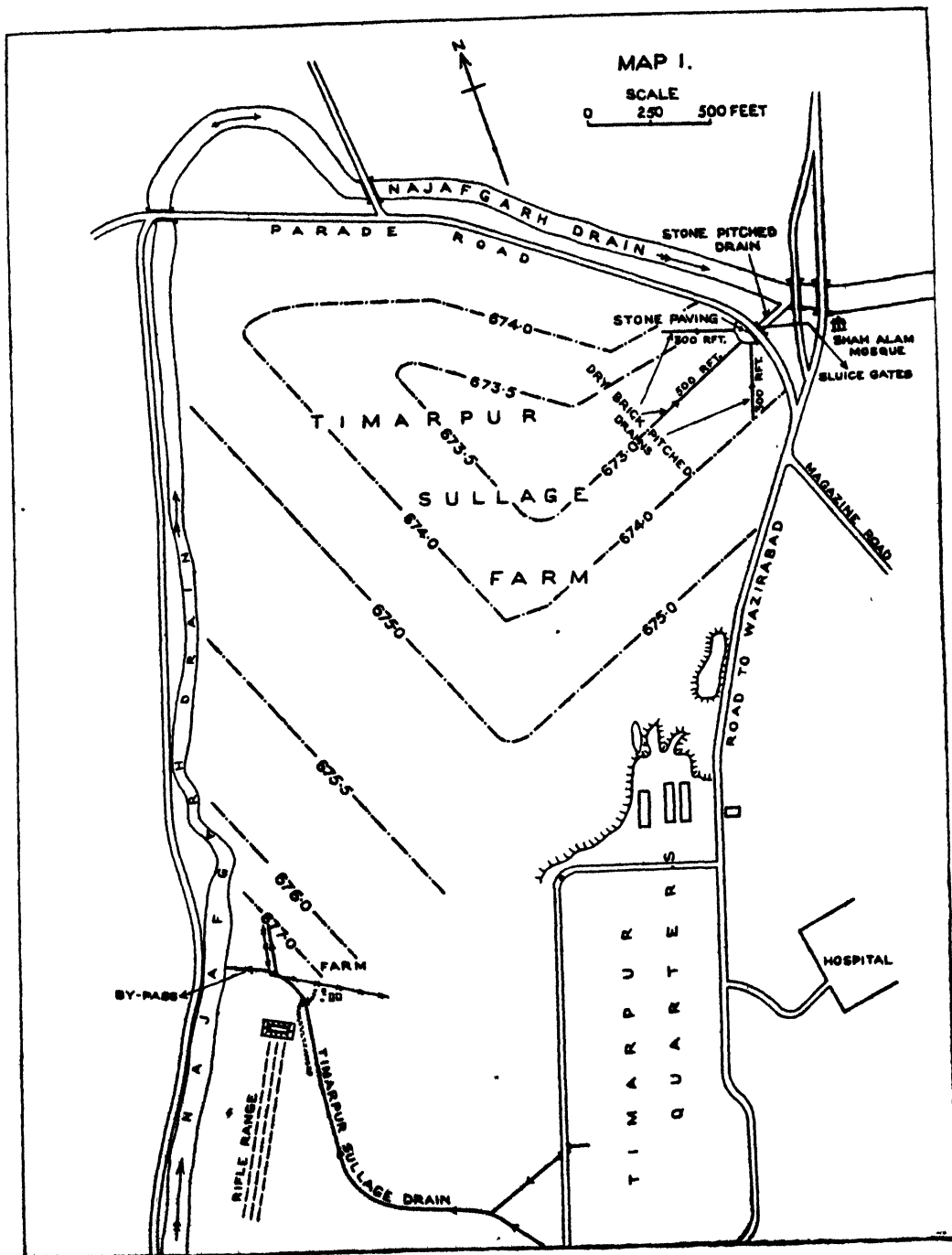
(4) *Upper Bela Road drains*.—There was an elaborate system of open stormwater drains extending from Metcalfe Nala to Jumna Railway Bridge, a distance of  $1\frac{1}{2}$  miles, which was actually constructed as an antimalaria work following Hodgson's survey of 1912-1913. Apparently the view then held was that drains should be dug as deep as possible, regardless of the resulting heading up of water when the river level rose. Consequently, for many weeks each year, these drains provided ideal breeding places for *A. culicifacies*. Some of them were entirely unnecessary, and have now been filled in, whilst the beds of the remainder have been raised by approximately 5 feet, so that their outfalls are as high as possible compatible with the provision of an adequate gradient. This level in each case corresponds to approximately R. L. 667 feet at the Power House, which is seldom exceeded for more than a few days in any one year (Table). The reconstruction of the Jumna Village drain, the most southerly

TABLE.

*River levels recorded at Electric Power House, 1932-1940.*

Feet above sea level.	Number of days in each year on which each level was reached.								
	1932	1933	1934	1935	1936	1937	1938	1939	1940
669	1	2	0	0	0	0	0	0	0
668	5	8	1	0	0	3	0	0	0
667	13	23	12	6	2	6	5	0	8
666	31	55	28	13	13	15	9	1	18
665	52	79	44	26	34	31	21	3	31







of the series, was particularly difficult, on account of the number of connections it received from the city. Many of these drains date from Moghul times, and their course is not shown in any existing plan. It was found necessary to provide well foundations for the outfall of this drain and for that of Qudsia Creek, owing to the presence of wet running sand.

(5) *Jumna Village ponds*.—The curious history of the origin of these ponds was told in Part I (Covell, *loc. cit.*). In order to fill them, it was necessary to construct a temporary bridge over the Jumna during the winter months to convey the earth across the river from the far bank.

(6) *Bela between Jumna Railway Bridge and Power House*.—This tract is bounded on the east by Jumna Creek, an arm of the river, and is traversed from west to east by two drains, the Fort Pukka Nala and the Khairati Nala, which divide it into three sections. Prior to 1936, all three sections were subject to river flooding to a greater or less extent each year.

A line of borrowpits alongside the Lower Bela Road has been filled in (Plate III). An old Moghul bund at the northern border of the tract has been repaired and has been continued along the whole length of Jumna Creek to the Power House, except where it is pierced by the two transverse drains. In these situations, it is joined by bunds which have been constructed along both sides of each drain (Plate IV). The height of the embankments in this area corresponds to a level of rather more than R. L. 670 feet at the Power House. As explained in Part I, previous records indicate that this is unlikely to be exceeded except on very rare occasions (about once in 20 years or so). The ground within them has been dressed so that no depressions remain, the slope being directed towards small sluice-gates which have been installed in the bunds of the drains, through which flood water may be discharged when the river level is sufficiently low. The bed of the Fort Pukka Nala was raised about 3 feet, and sullage water from the Fort, which formerly discharged into it, was diverted into a sewer, thus rendering the construction of a cunette unnecessary and effecting a considerable saving in consequence. The general level of the ground in the vicinity of Khairati Nala was too low to allow of any raising of the bed of this drain.

Both the Fort Pukka Nala and Khairati Nala have been fenced and provided with foot bridges, to prevent the depredations of cattle and damage from traffic.

Extensive filling operations, necessitating the use of a light railway, were required in the angle between the Power House Road and Lower Bela Road, which was formerly flooded through the frequent breaches which formerly occurred in the banks of the City Ditch (Plate V). The latter, a large open drain running along the southern border of the city, which formerly traversed this area, has been diverted, and its former bed filled in (*see below*).

(7) *The Bela from Power House to Purana Qila*.—The newly aligned City Ditch, after being diverted diagonally from the corner of the city and carried through a culvert beneath the Power House Road, now joins an underground stormwater drain, whose bed had to be raised for some distance for this purpose. The combined drain is carried to the river bank, the outfall being kept at as high a level as possible, as in the case of the Upper Bela drains (Plate VI). Extensive filling and levelling work was done between this drain and the Power House Road, the light railway again being used to convey the necessary earth (Plate VII).



In 1939, work was commenced on the embankment of a railway designed to bring coal to the Power House from the Agra-Delhi Chord line. This provided a golden opportunity for protecting another large section of the Bela from river flooding. In the ordinary course of events, a double line of large borrowpits would have been excavated to provide earth for the embankment, and these would have been filled with river water each year, just as in the case of the Agra-Delhi Chord line. In this case, it was arranged that the culverts piercing the new embankment should be closed, and the earth for the embankment taken only from such places as were indicated by the Malaria Department. On the river side of the embankment, the earth was cut in such a manner as to slope the ground towards the bank, along the toe of which a drainage channel was made. On the inner side of the railway, a drain was dug where this was required for the antimalaria scheme, and the earth so excavated used for the embankment. Additional earth for the embankment was provided by digging a large tank ('Lido' No. 2) which was also required for the antimalaria scheme. Not a single borrowpit was made during the whole course of the work.

The most southern section of this part of the Bela is traversed by two large high-level stormwater drains (Nos. 12 and 14), constructed as part of the antimalaria programme. The embankments flanking the outfalls of these have now been connected by a bund, in order to protect yet another section of the Bela from river flooding. The effect of this was to render unnecessary the pitching of the southern section of the Power House Railway embankment, thus saving the railway authorities some thousands of rupees.

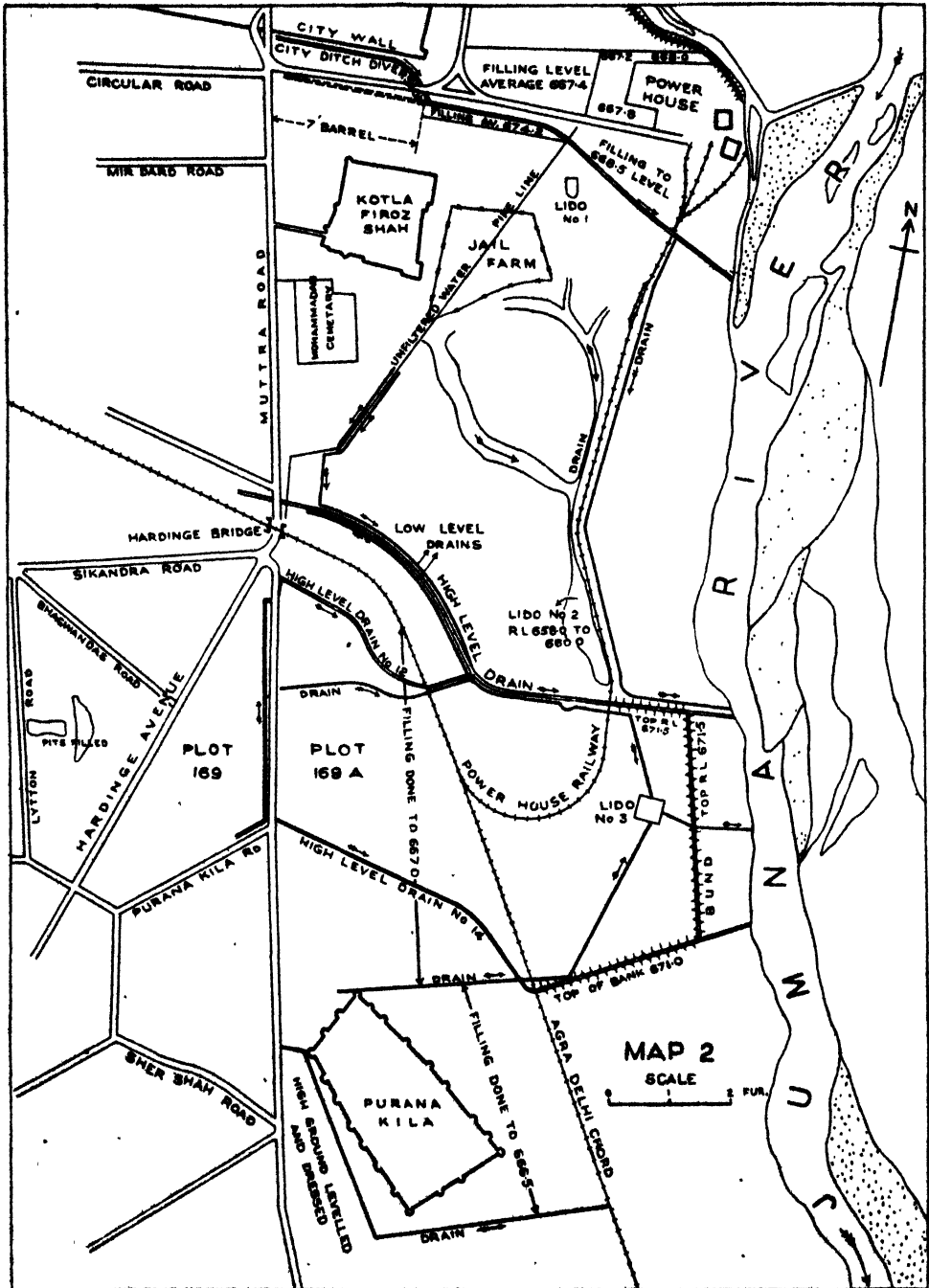
In this manner, a project, originally planned on purely utilitarian grounds, has assumed the character of an important sanitary work, as the result of intimate collaboration between the engineers and the Malaria Department, with material benefit to both parties concerned. It is believed that this is the first occasion on which such a result has been achieved in this country, and as such it constitutes an important landmark in the history of malaria control in India.

There remained the problem of dealing with rainwater precipitated on the very extensive area now protected from river flooding, but without any exit for local drainage. Three tanks, or 'lidos' (including that above referred to), have been excavated at the points shown in Map 2, and the ground so sloped that all local rainwater is directed into one or other of these. The principles followed in this technique, which was developed as the result of a special study by Major M. K. Afridi, I.M.S., are to take advantage of the natural slope of the ground wherever possible, to avoid long 'leads' for any filling which may be necessary and to construct no formal drains except where these are absolutely necessary. In the case here cited, there is only one short section of drain a few yards in length between the Power House Road and Drain 12, an area nearly 2 miles long and more than half a mile broad. This results in a great saving in maintenance charges; indeed the whole system can be kept in good repair without difficulty by the normal antimalaria gang, as part of their routine work.

A culvert was constructed at our request in the railway embankment opposite Lido No. 2, and another has been made in the bund connecting the embankment of Drains 12 and 14, opposite Lido No. 3. These culverts are normally closed by plank regulators packed with earth, and will only be brought into use in the event of floods caused by a breach in one of the embankments, or by abnormally heavy rainfall. Sluice-gates are not employed, because



## Antimalaria Operations in Delhi.





experience has shown that unless these are very small they are never absolutely water-tight.

Allusion has already been made to the double line of enormous borrowpits which existed along the course of the Agra-Delhi Chord Railway embankment, and which were filled each year by river flooding. Between the embankment and Hardinge Avenue was a low-lying area formerly traversed by an arm of the Jumna River and divided by the Muttra Road into two sections, known as Plots 169 and 169A. Every year the river flood water used to extend as far as the Muttra Road, and the whole area was a prolific source of breeding of *A. culicifacies*. Very extensive filling operations were done in both these areas as part of the antimalaria works programme, the light railway being used for this purpose. Plot 169 has provided very valuable building sites, which are now in course of development. The great borrowpits alongside the railway embankment have been filled up to a point some distance south of Purana Qila (Plate VIII). The high level stormwater drains (12 and 14) above referred to are carried across Plot 169A, the surface drainage of the plot itself being provided for by low level drains discharging into Lido No. 3 (Map 2).

## B. SOUTHERN AREA.

(1) *Canalization of Barapula Nala*.—This channel carries the discharge from an underground stormwater drain issuing from the new city area at Q point. It also receives stormwater from the great drain formed by the junction of the Kushak and Chiragh Dehli Nalas referred to below. An enormous volume of water flows down the Barapula Nala after heavy rainfall, for the catchment area served by it includes the whole of the Southern Ridge, the aerodrome, and an extensive tract of the country lying to the south of New Delhi. A central channel has been constructed to take the normal flow, the cunette proper being cemented, whilst the sides are lined with dry brick pitching. Outside this, the bed of the channel and its embankments are unlined, but are covered by a natural growth of grass (Plates IX and X).

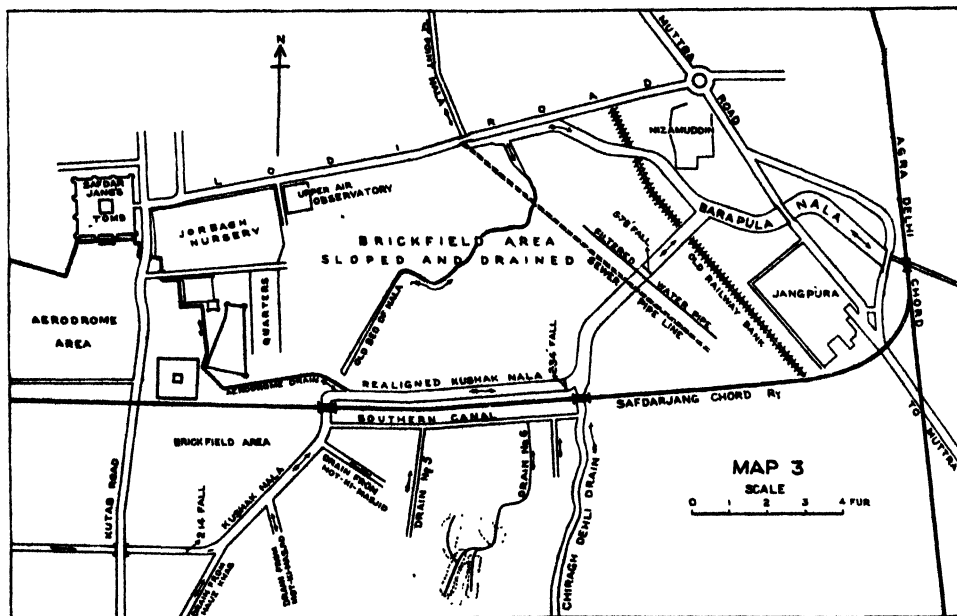
(2) *Drainage of Nizamuddin and Jangpura*.—These are two villages within the urban area, for which no proper surface drainage existed. A system of unlined drains has now been provided to carry stormwater into Barapula Nala.

(3) *The Brickfields Area and Kushak Nala*.—The Kushak Nala is the great stormwater drain which runs along the foot of the Southern Ridge and sweeps round in a curve until it finally follows a north-easterly course towards Barapula Nala. The catchment area served by this drain covers  $15\frac{1}{2}$  square miles. Its lower section was not previously canalized, so that after heavy rainfall stormwater flooded a large area immediately south of New Delhi, filling the innumerable pits scattered over the abandoned brickfield sites, which continued to hold water throughout the greater part of the year. A stormwater drain from the aerodrome also discharged in a haphazard fashion into brickfields area.

The Kushak Nala has now been re-aligned and canalized as part of the antimalaria works programme and now runs parallel with, and immediately south of, the Quarry Railway, to join with the Chiragh Dehli Nala just after the latter has crossed beneath the line. From this point the combined channel, the bed of which is 200 feet in width, runs in a north-easterly direction, to join Barapula Nala. A central cunette has been provided similar to that in Barapula Nala. The aerodrome drain now discharges into Kushak Nala immediately after the latter has crossed beneath the Quarry Railway (Map 3).



The brickfields area having been thus protected from flooding by extraneous water, the next problem was to arrange for adequate local drainage. It was at first intended to treat the area in the same way as the Bela south of the Power House, i.e., by dressing the ground so that all the water would drain into one or more 'lidos'. As the work proceeded, however, it became apparent that, by a slight modification in the original plan, all the water could be discharged through a channel representing the former course of the Kushak Nala. This has now been done, and has proved completely satisfactory, all local rainwater draining away in a few hours' time (Plate XI). Several lacs of rupees originally budgeted for major operations in this area have been saved by the adoption of this method.



### C. THE RIDGE.

As already explained, the first portion of the Kushak Nala was designed to prevent rainwater falling on the Southern Ridge from inundating the site of the new capital. In two situations, the drain crosses the lower ends of ravines, the beds of which were at a considerably lower level than that of the drain itself, so that water in them could not drain away. These have now been filled as a part of the antimalaria works programme. Some idea of their extent can be gained from the fact that no less than 1½ million cubic feet of earth were required to fill them. In places, the filling exceeded 14 feet in depth.

No attempt has been made to deal with the vast number of abandoned quarry pits in the Northern and Southern Ridges by permanent measures, for mosquito breeding in them can be effectively controlled by systematic oiling, and the cost of filling them would be prohibitive under present circumstances. The system of controlled quarrying now in force in the Delhi urban area was described in Part II.



PLATE I.  
AREA NORTH-EAST OF TIMARPUR SULLAGE FARM.



Fig. 1. Before treatment



Fig. 2. After treatment.



PLATE II.  
DEPRESSION SOUTH OF METCALFE NALA BUND.

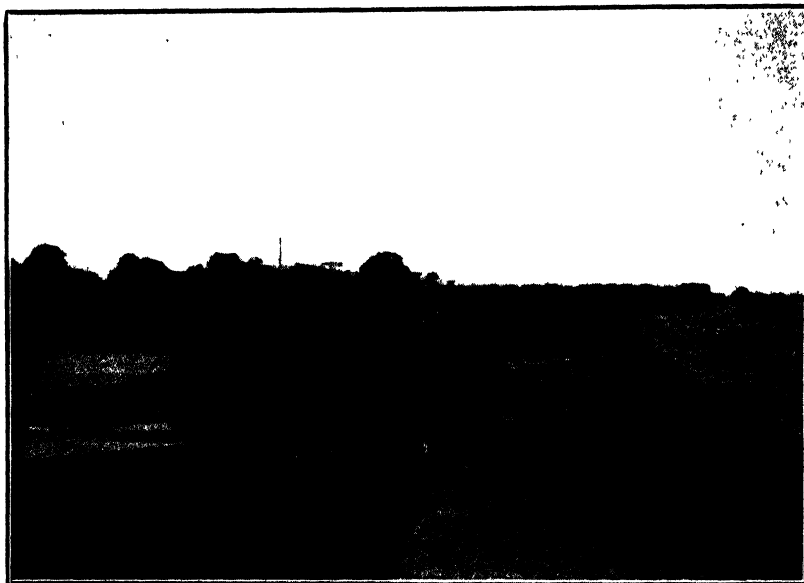


Fig. 3. Before treatment.



Fig. 4. After treatment.



PLATE III.  
BORROWPITS ALONG LOWER BELA ROAD.



Fig. 5. Before treatment.



Fig. 6. After treatment.



PLATE IV.  
FORT PUKKA NALA.



Fig. 7. Before treatment.

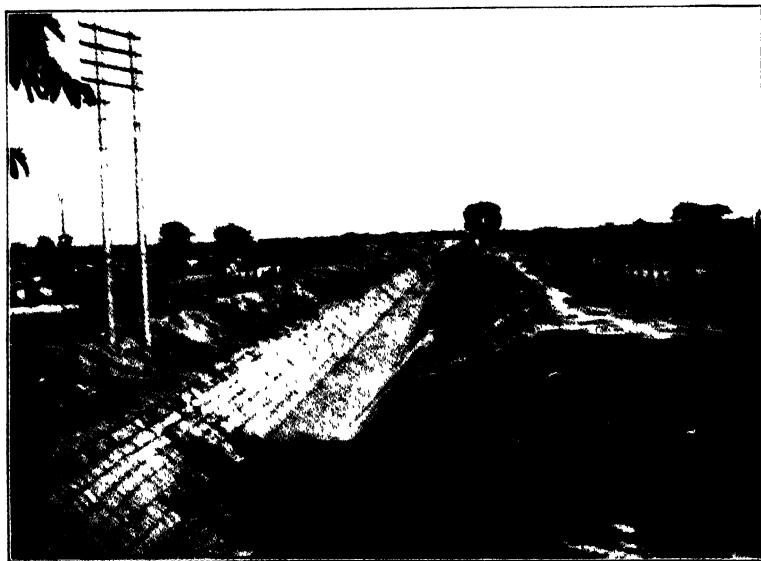


Fig 8. After treatment (drain fenced, bed raised, banks raised, sluice-gate and foot-bridge provided).



PLATE V.  
BORROWPITS AT JUNCTION OF LOWER BELA ROAD AND POWER HOUSE ROAD.



Fig. 9. Before treatment.



Fig. 10. After treatment.



PLATE VI.  
BELA SOUTH OF POWER HOUSE ROAD, LOOKING WEST.



Fig 11. Before treatment

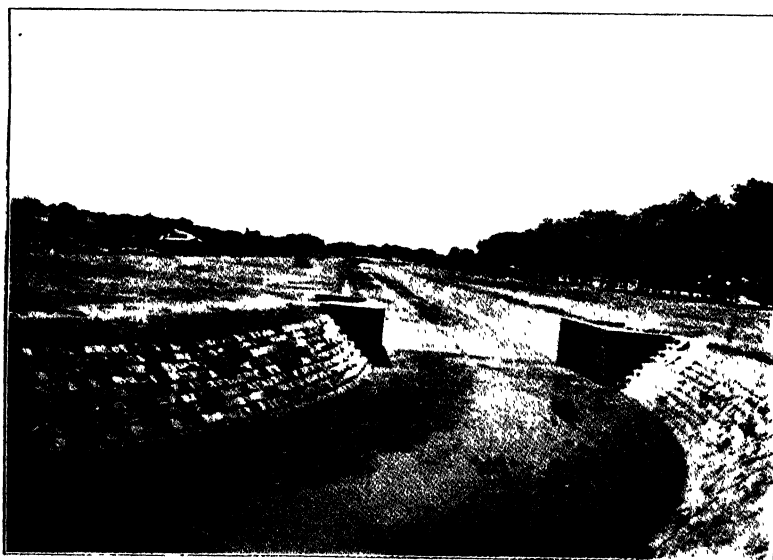


Fig. 12. After treatment.



PLATE VII.  
BELA SOUTH OF POWER HOUSE ROAD, LOOKING EAST.



Fig. 13 Before treatment



Fig. 14. After treatment.



PLATE VIII.  
BORROWPITS WEST OF AGRA-DELHI CHORD RAILWAY EMBANKMENT.

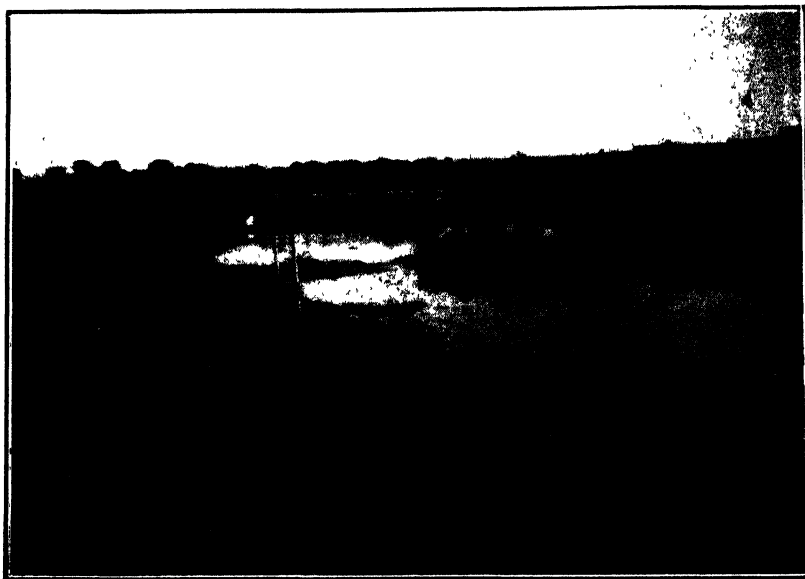


Fig 15. Before treatment.



Fig. 16. After treatment.



PLATE IX.  
BARAPULA NALA ABOVE BARAPULA ROAD BRIDGE.



Fig. 17. Before treatment.



Fig. 18. After treatment.



PLATE X.  
BARAPULA NALA BELOW BARAPULA ROAD BRIDGE.



Fig. 19. Before treatment.



Fig. 20. After treatment.



PLATE XI.  
BRICKFIELD AREA.



Fig. 21. Before treatment.



Fig. 22. After treatment.



PLATE XII.  
WATER-LOGGED AREA WEST OF SABZIMANDI RAILWAY STATION.

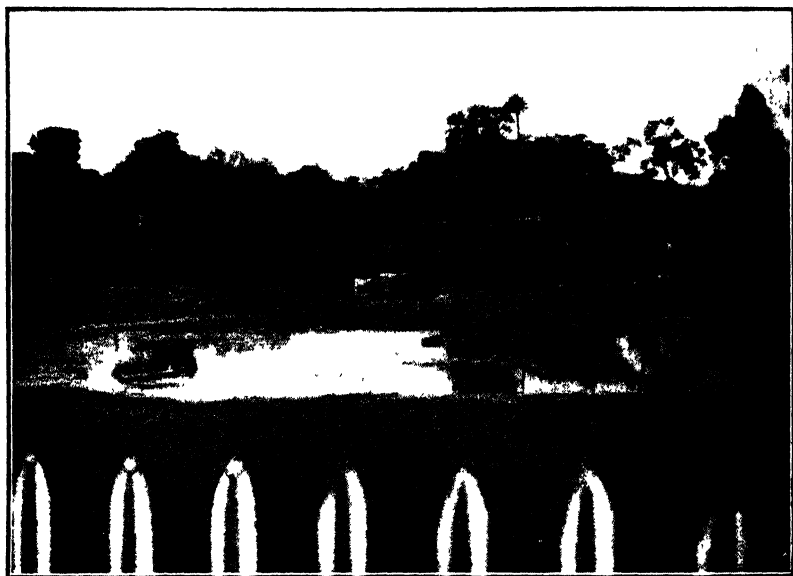


Fig. 23. Before treatment.

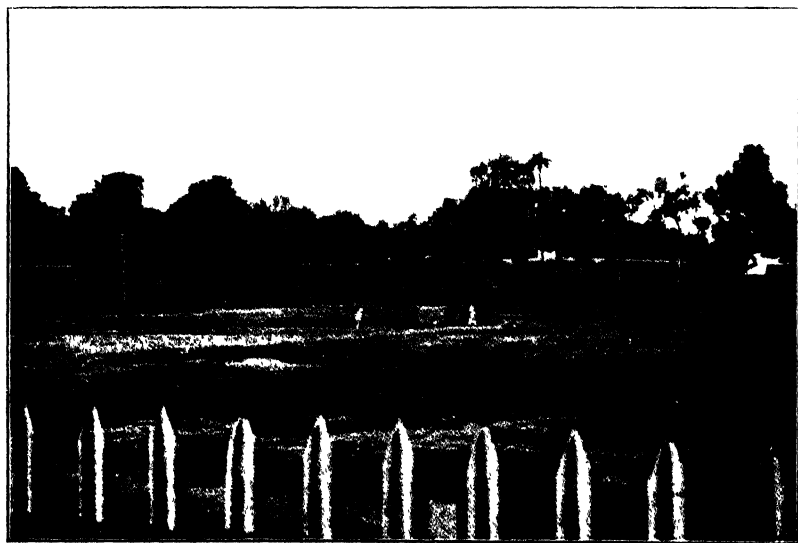


Fig. 24. After treatment.



PLATE XIII.  
NAJAFGARH CUT IN FRUIT GARDEN AREA.



Fig. 25. Before treatment.

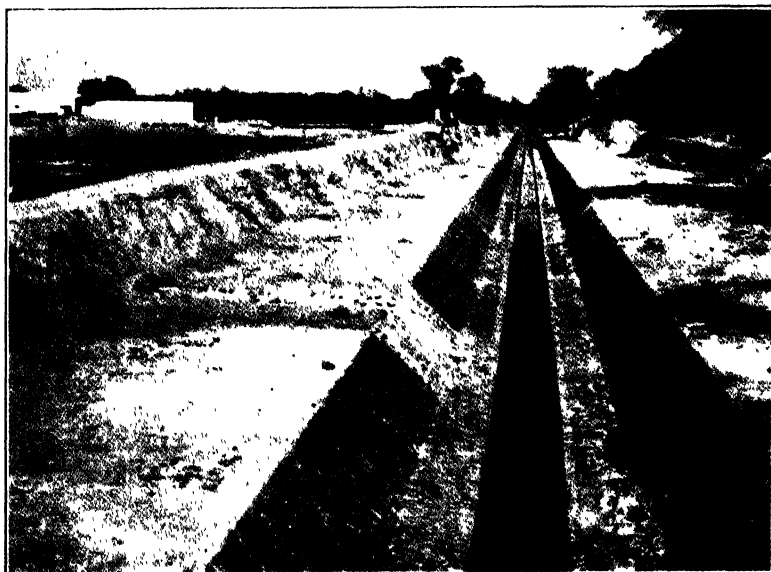


Fig. 26. After treatment.



PLATE XIV.  
DARHALIA ESCAPE, SOUTH OF LOWER ROHTAK ROAD.



Fig 27. Before treatment.



Fig. 28. After treatment.



#### D. THE CANAL-IRRIGATED AREA.

(1) *Western Jumna Canal closure scheme.*—This was one of the major items on the programme of permanent works and funds for it were sanctioned in December 1936, but the execution of the project has been held up pending the settlement of an unfortunate dispute regarding the ownership of the land traversed by the canal.

The effect of the scheme will be : (i) closure of the canal a short distance upstream of Andha Moghul Bridge, opposite Sabzimandi Railway Station; (ii) filling the original bed of the canal below the point of closure; (iii) provision of an alternative piped water supply from the Jumna to serve areas which will thus be deprived of canal water; (iv) lining and fencing the canal from its point of intersection with the Najafgarh Cut to its termination, the width and depth of this section being at the same time greatly reduced; (v) re-grading and lining the watercourses originating in this reach; and (vi) curtailment of the amount of water supplied to the local fruit gardens.

The measures which are being employed to control mosquito breeding and larval drift in this area pending the execution of the scheme were described in Part II.

(2) *Railway area near Sabzimandi Station.*—Extensive low-lying areas on both sides of the line, which were formerly flooded with water through leakage from the canal irrigation system, have been filled and dressed, local rainwater being carried by drains to Darhalia Drain (Plate XII).

(3) *Canalization and stone-pitching of Darhalia Drain, and part of Najafgarh Cut.*—As the result of this work the whole of Darhalia Drain (3½ miles in length) has now been canalized and pitched (Plate XIII). The work has been continued down the Najafgarh Cut below its junction with Darhalia Drain, as far as the portion which was already pitched (Plate XIV). In the latter portion, the original pitching of the sides of the channel has been raised to a height above the normal high flood level. Below this point the work is now being carried on as far as the tomb of Shah Alam, which is situated about 200 yards above the outfall of the Cut into the River Jumna.

#### E. CENTRAL AREA.

In this area a very large number of pits have been filled, some of them of enormous size, in particular the railway borrowpits east of New Delhi Station, the disused section of Q point Nala, and two great depressions west of Hardinge Avenue.

Other works include the replacement of a number of road siphons by properly graded culverts, the mosquito-proofing of cisterns, the filling or covering of wells, and a number of modifications in the underground stormwater drainage system. In the case of the last mentioned work, all connections with road gratings are now provided with offsets in the form of a horizontal pipe. The minimum length of the offset is 12 feet for a 6-inch pipe, and 18 feet for a 9-inch pipe. Where open stormwater drains discharge into this system, their terminal portion is covered for a distance depending on the area of the cross-section of the drain.

#### COSTS.

A list of the various permanent antimalaria works and the cost of each is given in the Appendix. The total amount already spent is approximately



Rs. 15 lacs (£113,400). The original estimate for the Western Jumna Canal closure scheme, the only item of the programme now outstanding, was Rs. 1·82 lacs; but this sum did not include the provision of an alternative water supply, and the final cost is expected to be approximately Rs. 3 lacs.

The total cost of the permanent engineering works will thus be in the neighbourhood of Rs. 18 lacs (£135,000), which represents about one per cent of the cost of the new capital. At least 25 per cent of this expenditure has, however, been incurred for works designed solely for the protection of the old city and its extensions, and should not, therefore, be debited to the cost of New Delhi. The population of the whole urban area is now about 500,000, so that the capital expenditure on antimalaria works amounts to approximately Rs. 3·8-0 per head.

When the original programme of antimalaria works was planned, it was expected that the expenditure involved would be in the neighbourhood of Rs. 30 lacs (£225,000). The reduction which has been effected is chiefly due to the development of the special methods described above, particularly in the case of the brickfield area and the Bela south of the Electric Power House, in each of which there was a saving of approximately Rs. 5 lacs. In some instances, valuable land has been reclaimed for building sites, whilst in many others the value of property has been greatly enhanced, on account of the improvement in health conditions. A single plot (No. 169) in New Delhi is expected to bring in Rs. 5 lacs when sold for building purposes, besides an annual sum of Rs. 25,000 as ground rent. It is difficult to estimate how much money has been saved to industry by the fall in malaria incidence which has been effected, but it may be stated with certainty that the cost of permanent antimalaria works in Delhi has already been amply repaid by the results achieved.

#### THE FUTURE OF MALARIA CONTROL IN DELHI.

The importance of avoiding the creation of malarial conditions during the future development of the Delhi urban area was fully recognized by the New Delhi Development Committee (1939), one of whose recommendations reads as follows :—

‘For all extensions in undeveloped areas, antimalaria works will be required. These include the completion of the original antimalaria programme: the provision of the usual half-mile protective belt round the extensions: and the levelling and clearing of all building sites before development begins’. The Committee recommended the formation of a standing committee, on which the Director, Malaria Institute of India, would serve, to co-ordinate the activities of the various authorities concerned with the development of New Delhi and its surroundings. This Committee has now been constituted by the Government of India. There is thus no likelihood of any future reversion to the conditions which obtained prior to the inception of the present antimalaria campaign.

#### SUMMARY.

(1) Details are given of the permanent engineering works carried out in the Delhi urban area since 1936. The programme has now been completed, with the exception of one major project (see para 6 below).

(2) The general principle adopted was to prevent flooding from any source over the whole inhabited area and for at least half a mile beyond its limits. The area so protected is levelled and dressed in such a manner as to drain away



local rainwater completely, or, where this cannot be effected, to direct it into one or more tanks (*hidros*) specially excavated for the purpose. The adoption of this technique, which aims at the reduction of filling 'leads' and formal drains to an absolute minimum, has led to a substantial reduction in the cost of the antimalaria programme.

(3) In certain sections, located in the midst of, or in close proximity to, built up areas, extensive filling operations have been carried out. These have in many instances provided valuable building sites, and in other cases have greatly enhanced the value of house property.

(4) Heading up of water in stormwater drains discharging into the river has been reduced to a minimum by raising their outfalls to the highest level compatible with a gradient sufficient to secure an adequate flow.

(5) Other works include the remedying of various defects in the underground stormwater drainage system, the mosquito-proofing of cisterns, the filling or covering of wells, and the abolition of road siphons.

(6) A scheme for lining the lower reach of the Western Jumna Canal tail distributary and its branches, and for closing its terminal section was sanctioned in December 1936, but its execution has hitherto been postponed owing to certain administrative difficulties.

(7) The cost of the works already completed is approximately Rs. 15 lacs (£113,400). The final figure will be approximately Rs. 18 lacs (£135,000), representing about one per cent of the cost of construction of the new capital. Some 25 per cent of the outlay is, however, debitable to works designed to protect the old city of Delhi and its western and north-western extensions. The development of special methods of drainage technique during the past 5 years has resulted in a saving of more than Rs. 10 lacs (£75,000) on the amount originally estimated to complete the antimalaria works programme.

(8) Adequate control of malaria throughout the urban area in future will be secured if the recommendations of the New Delhi Development Committee (*loc. cit.*) are implemented in full. A standing committee, of which the Director, Malaria Institute of India, is a member, has recently been formed to co-ordinate the activities of the various authorities concerned with the development of New Delhi and its surroundings.

## REFERENCES.

- COVELL, G. (1939) .. .. Antimalaria operations in Delhi. Part I. *J. Mal. Inst. Ind.*, **2**, 1, pp. 1-61.
- COVELL, G., and AFRIDI, M. K. (1939). Antimalaria operations in Delhi. Part II. *Ibid.*, **4**, pp. 315-340.
- NEW DELHI DEVELOPMENT COM- Report, Govt. Press, Simla.  
MITTEE (1939).



## APPENDIX.

## CAPITAL COST OF PERMANENT ANTIMALARIA WORKS IN DELHI URBAN AREA\*.

## A. COMPLETED WORKS.

	Rs.
Filling and draining Block 169 (between Hardinge Avenue, Muttra Road and Purana Qila Road) .. ..	7,09,946
Filling and draining area east of Muttra Road, from Hardinge Avenue Bridge to Purana Qila .. ..	835
Demolition of Power House irrigation channel .. ..	1,543
Filling borrowpits east of Ferozeshah Kotla .. ..	5,202
Filling disused part of Q point Outfall Nala .. ..	29,995
Filling depressions in Jumna Village .. ..	46,863
Raising beds of stormwater drains between Metcalfe House and Jumna Railway Bridge .. ..	12,883
Filling pits near Metcalfe House and Metcalfe Nala Outfall .. ..	4,430
Filling depressions near Metcalfe House .. ..	5,770
Filling depressions west of Paharganj .. ..	19,428
Filling excavations between Lytton Road, Hardinge Avenue and Bhagwan Dass Road .. ..	31,165
Canalization and cunetting of Q point Outfall Drain from Lodi Bridge to railway bridge south of Nizamuddin .. ..	75,377
Diversion of City Ditch and filling of pits along Lower Bela and Power House Roads .. ..	29,605
Raising and pitching bed of terminal portion of City Ditch and filling area between this and Power House Road .. ..	860
Petty works in Bela opposite Fort .. ..	5,942
Modification of road siphons .. ..	4,839
Mosquito-proofing of cisterns .. ..	6,879
Drainage of Timarpur Sullage Farm area .. ..	22,903
Filling pits in Paharganj and Kishanganj .. ..	1,46,302
Canalization and stone-pitching of Darhalia Escape and part of Najafgarh Cut .. ..	24,548
Modification of the New Delhi stormwater drainage system .. ..	17,337
Filling borrowpits between New Delhi Station and Hardinge Avenue Railway Bridge .. ..	21,389
Filling and draining ravines in Southern Ridge .. ..	25,790
Surface drainage of Nizamuddin and Jangpura villages .. ..	15,301
Closing wells in New Delhi .. ..	90,208
Extension of stormwater drains 12 and 14 to river bank, connecting these with a bund and dressing area between them .. ..	41,509
Raising bed of Jumna Village drain and covering this .. ..	1,14,750
Canalization of Kushak Nala .. ..	
Total .. ..	15,11,599

\* Excluding departmental charges.



**B. WORK SANCTIONED, BUT NOT YET TAKEN IN HAND.**

Closing of Western Jumna Canal Tail Distributary near Andha Moghul Bridge, filling its bed below this point, lining the distributary and its branches above this as far as the Najafgarh Cut, and provision of an alternative water supply for certain areas ..	Rs.
..	Approx. 3,00,000







## SPECIFIC AGGLUTINOGENIC PROPERTIES OF INACTIVATED SPOROZOITES OF *P. GALLINACEUM*\*.

BY

PAUL F. RUSSELL,

(*Field Staff, International Health Division of the Rockefeller Foundation*),

MAJOR H. W. MULLIGAN, I.M.S.

(*Director, Pasteur Institute of Southern India, Coonoor*),

AND

BADRI NATH MOHAN

(*Senior Technical Assistant, Malaria Investigations*).

[February 4, 1941.]

### INTRODUCTION.

IN a previous communication, Mulligan, Russell and Mohan (1940) reported that normal serum and heterologous malarial sera did not agglutinate sporozoites in saline dilutions above 1/128, whereas sera from fowls, sparrows and human patients with chronic malarial infections agglutinated sporozoites of the homologous *Plasmodium* in saline dilutions ranging from 1/1,000 to 1/65,000. The conclusion was reached that sporozoite agglutination in high dilutions of malarial sera was a specific reaction.

This paper reports : (1) the use of ultra-violet radiation as an effective means of rendering sporozoites of *P. gallinaceum* non-infective to susceptible fowls, and (2) the production of specific agglutinins in the sera of fowls after repeated injections of sporozoites inactivated by ultra-violet radiation.

### MATERIALS AND METHODS.

The strain of *P. gallinaceum* used was the same as that employed in previous work by the authors (*loc. cit.*). This strain was pathogenic for local

---

\* These studies were made under the auspices, and with the support, of the International Health Division of the Rockefeller Foundation, co-operating with the Pasteur Institute of Southern India. The authors are indebted to P. Balarama Menon, of Malaria Investigations, for technical assistance.



domestic fowls which were used exclusively as vertebrate hosts. Three species of mosquitoes were used to obtain the complete exogenous cycle of *P. gallinaceum*—*Aedes albopictus*, *Armigeres obturbans*, and *Armigeres kuchingensis*. A plentiful supply of each species was always available from laboratory colonies maintained for the purpose. Heavy sporozoite infections in the glands of each species were readily obtained after incubation periods of 11 to 14 days in an insectary in which temperature was maintained between 21° and 30°C. (70° and 85°F.) and relative humidity between 75 and 85 per cent.

Sera for agglutination tests were obtained from fowls in the usual manner, and all dilutions were made with normal saline solution. In carrying out these tests, it was found convenient to double the dilution of serum in serial tubes and to provide each tube with a separate clean pipette. After dissections of the salivary glands of infected mosquitoes had been made in saline dilutions of serum or in normal saline solution, the preparations were covered with a 6 mm. square coverslip before examination. Preparations which failed to show heavy sporozoite infections were discarded and replaced. Examination for the presence of sporozoite agglutination was carried out at a magnification of approximately 200 diameters. All readings were recorded within 15 minutes of dissection.

Great variations in degree of agglutination were observed with different sera in varying dilutions. The same five arbitrary classes of agglutination were used as have been described by the authors in their previous paper (*loc. cit.*). The chief characters of these five grades of agglutination are given below but, for a fuller description, the previous paper should be consulted.

(1) *Agglutination* +++++. Very large dense masses of sporozoites, often in the form of rosettes or groups of rosettes: clumps of several hundreds of sporozoites, including rosettes, apparent in less than 5 minutes after dissection.

(2) *Agglutination* ++++. Large dense masses of sporozoites, often in the form of discrete rosettes, apparent within 5 minutes of dissection.

(3) *Agglutination* ++++. Large aggregations of sporozoites, but never in the form of rosettes: clumps of sporozoites, frequently arranged in a characteristic 'wire-netting' pattern, apparent within 5 to 10 minutes after dissection: free discrete sporozoites more numerous than in (1) and (2) above.

(4) *Agglutination* ++. Clumps of 10 or 12 sporozoites present within 10 minutes: smaller clumps numerous: free discrete sporozoites abundant.

(5) *Agglutination* +. Unmistakable agglutination of less intensity than any of the above classes: clumps of 5 or 6 sporozoites present within 10 to 15 minutes of dissection.

Dissections in normal saline solution were used as controls. In every case, gentle pressure was applied to the coverslip to disperse sporozoites which tended to form false clumps by reason of excessive numbers. Sporozoites which were clumped by agglutination could not be dispersed as free discrete bodies in this way. Agglutination of sporozoites was best read in microscopic fields at some little distance from dissected gland tissue, thereby avoiding examination of fields with closely 'packed' sporozoites. It was found advisable to use only heavily and consistently infected mosquitoes in these studies.

Sporozoites inactivated by the method to be described were dissected in normal saline solution, exposed to ultra-violet radiation, diluted with saline solution, drawn into a tuberculin syringe and injected intravenously or intramuscularly into fowls.



## USE OF ULTRA-VIOLET LIGHT TO INACTIVATE SPOROZOITES.

It was found possible to inactivate sporozoites by exposure to ultra-violet radiation. For this purpose a 'Homesun' sun lamp\* was employed. This lamp has an S. 250 electronic discharge quartz tube mercury arc, and is rated to give 3,200 Angström units at 40 inches. Salivary glands, heavily infected with sporozoites, were dissected in normal saline solution, placed in shallow watch glasses, and exposed to the direct rays of the lamp at a distance of 16 inches from the mercury arc. The amount of saline solution used in dissection was reduced to a minimum.

TABLE I.

*Effect of exposure to ultra-violet radiation on the infectivity of sporozoites of P. gallinaceum.*

Number of mosquitoes dissected.	Exposure to ultra-violet lamp in minutes.	Route of injection of sporozoites into fowl.†	Number of experiments.	Result.	Serial numbers of fowls.
10	3	i/m	2	Infection	85, 103.
10	5	i/m	2	Negative	86, 104.
10	10	i/m	1	"	87.
10	15	i/m	2	"	79, 80.
10	15	i/v	8	"	93-99, 105.
10	30	i/m	2	"	81, 82.
20	15	i/m	4	"	79, 81, 82, 86.
20	15	i/v	4	"	93, 94, 96, 99.
20	20	i/m	8	"	79-82 (each twice).
30	20	i/v	3	"	93, 94, 96.
30	30	i/v	1	Infection	119 ( <i>see text</i> ).
30	30	i/v	4	Negative	120, 121, 125, 126.
37	30	i/v	1	"	104.
40 *	20	i/v	1	Infection	104 ( <i>see text</i> ).
40 †	30	i/v	51	Negative	120, 121, 125, 126 (each four times). 123, 124, 127, 128, 148-150 (each five times).

\* In two batches of 20, each exposed to radiation for 20 minutes.

† In four batches of 10, each exposed to radiation for 30 minutes.

‡ i/m = intramuscular. i/v = intravenous.

\* Manufactured by Hanovia, Slough, England.



Different numbers of salivary gland dissections were exposed to ultra-violet radiation for varying periods of time, as shown in Table I. Exposure of the glands of 10 mosquitoes to the action of the lamp for 3 minutes did not inactivate sporozoites, as proved by their subsequent infectivity for susceptible fowls (85 and 103). Exposure to the lamp's rays for 5 minutes or longer appeared, in most cases, to inactivate sporozoites. In fowl 119, infection resulted after the injection of the gland dissections of 30 mosquitoes exposed to the lamp for 30 minutes, and in fowl 104 infection followed the injection of 40 gland dissections which had been exposed to radiation in two equal parts for 20 minutes.

TABLE II.

*Agglutinating titre of normal fowl serum for sporozoites of P. gallinaceum.*

Fowl number.	1/2	1/4	1/8	1/16	1/32	1/64	1/128	1/256	1/512
1	+++	+	+	+	—	—	..	..	..
2	+++	++	+	+	—	—	..	..	..
3	++++	+++	+++	+++	+++	+	+	—	—
4	++++	++++	+++	++	+	—	—	—	..
5	+++	+++	+	—	—	—	..	..	..
120	..	++	+	+	+	—	..	..	..
121	..	+	+	—	..	..	..	..	.
123	..	+++	+++	++	+	+	—	..	..
124	..	+++	++	++	+	+	+	+	—
125	..	++	++	+	—	..	..	..	..
126	..	++++	+++	+++	++	+	+	+	—
127	..	++++	+++	++	+	+	—	—	—
128	..	+++	+++	++	++	+	+	—	—
148	..	+++	+++	++	+	+	—	—	..
149	..	+++	+++	++	++	+	+	—	..
150	..	+++	+++	++	++	+	+	—	—

*Notes.*—See text for explanation of + symbols.

— means that no agglutination was observed.

In one of these fowls (104), there was a possibility of subsequent contamination by live sporozoites during manipulation, while in the other (119), failure to inactivate may have been due to inadequate penetration of ultra-violet rays through the relatively large amount of saline solution in the watch glass. The



technique was finally standardized at 30 minutes' exposure of the glands of not more than 10 mosquitoes in one watch glass placed at 16 inches from the mercury arc, using not more than 0.25 c.c. of saline solution for each lot of 10 dissections. No infection resulted in 51 consecutive tests using this standardized technique. The exposure employed was probably considerably longer than necessary to inactivate sporozoites, but it was desirable to allow a considerable margin of safety, especially as it appeared from the length of exposure required to produce erythema of the human skin that lamps of the type used tend to diminish in intensity of radiation after continued use. Some slight evaporation of saline solution was observed during the longer exposures to ultra-violet radiation.

Dissections of infected salivary glands in normal saline solution, not irradiated, were proved to be infective for normal fowls for periods extending up to 45 minutes after dissection. The possibility of their viability after longer periods was not investigated.

### INJECTIONS OF INACTIVATED SPOROZOITES.

The agglutinating titres of the sera of 16 normal fowls for sporozoites of *P. gallinaceum* are shown in Table II. Normal fowl serum did not agglutinate sporozoites in saline dilutions exceeding 1/256, and, as a rule, the titre was not above 1/64. In one case, the highest dilution at which agglutination occurred was only 1/8 (fowl 5), and in 3 other cases (fowls 1, 2 and 125) it was only 1/16. The results of agglutination tests on 19 fowls which received repeated injections of sporozoites inactivated by ultra-violet light are shown in Table III. In the first 8 fowls listed in Table III (fowls 79 to 82, 93, 94, 96 and 104), the agglutinating titre of the serum was not ascertained before injection, but there is little doubt that, after repeated injections, it was appreciably raised above the normal level in every case (cf. Table II). Fowls 79 to 82 each received four intramuscular injections of inactivated sporozoites obtained from batches of 10, 20, 20 and 20 heavily infected mosquitoes. In every case, the agglutinating titre of the serum of these fowls was at least 1/1,024, and in one case (fowl 82) it was 1/16,384. Fowls 93, 94 and 96 each received three intravenous injections of inactivated sporozoites from batches of 10, 20 and 30 heavily infected mosquitoes. In no case was the agglutinating titre of the serum less than 1/4,096, and in one case it was 1/262,144.

The remaining 11 fowls in this series (fowls 120, 121, 123 to 128, 148 to 150) each received intravenous injections of inactivated sporozoites at weekly intervals. The numbers of gland dissections injected were obtained from batches of 40 (occasionally 30 at the first injection), 40, 40, 40 and 40 heavily infected mosquitoes, except in fowls 148 and 149 which received only three injections. Lots of 10 gland dissections were exposed to ultra-violet radiation for 30 minutes. In every case, the agglutinating titre of normal fowl serum was ascertained before injections of sporozoites were commenced, a second agglutination test was carried out one week after the third injection and a third estimation was made 5 to 7 days after the fifth injection. In 2 cases (fowls 124 and 126), the normal serum agglutinated up to a titre of 1/256, while in one case (fowl 121) there was no agglutination in dilution above 1/8.

In every case, a considerable rise in agglutinating titre was observed after three injections of inactivated sporozoites (Table V). The least response was











TABLE IV.

*Agglutinating titre of sera of fowls infected with P. gallinaceum by blood inoculation.*

Fowl number	Stage of infection.	AGGLUTINATING TITRE OF FOWL SERA.											
		1/2.	1/4.	1/8.	1/16.	1/32.	1/64.	1/128.	1/256.	1/512.	1/1024.	1/2048.	1/4096.
6	Acute	+++	++	++	++	+	+	:	:	+	+	+	+
7	"	+++	++	++	++	++	++	+	+	+	+	+	+
8	Chronic	+++	+++	+++	+++	+++	+++	++	++	++	+	:	:
9	"	+++	+++	+++	+++	+++	+++	++	++	++	++	+	:
10	"	+++	+++	+++	+++	+++	+++	++	++	++	++	++	+
11	"	+++	+++	+++	+++	+++	+++	++	++	++	++	++	+
12	"	+++	+++	+++	+++	++	++	++	++	++	+	+	+
13	"	+++	+++	+++	+++	++	++	++	++	++	++	+	+
													1/262144.
													1/131072.
													1/65536.
													1/32768.
													1/16384.
													1/8192.

Notes.—See text for explanation of + symbols.  
— means that no agglutination was observed.



TABLE V.

*Increase in agglutinating titre of fowl sera after injections of inactivated sporozoites of P. gallinaceum.*

Fowl number.	HIGHEST AGGLUTINATING TITHE.			INCREASE IN NUMBER OF DILUTION TUBES.		
	Before injection (normal).	After three injections.	After five injections.	After three injections.	After five injections.	Difference between three and five injections.
120	1/32	1/1024	1/16384	5	9	4
121	1/8	1/2048	1/16384	8	11	3
123	1/64	1/2048	1/16384	5	8	3
124	1/256	1/512	1/32768	1	7	6
125	1/16	1/16384	1/131074	10	13	3
126	1/256	1/65536	1/262144	8	10	2
127	1/64	1/1024	1/65536	4	10	6
128	1/128	1/4096	1/131074	5	10	5
148	1/64	1/512	Died *	3	..	..
149	1/128	1/4096	Died *	5	..	..
150	1/128	1/512	1/32768	2	8	6

Notes.—This table is based on Table III above.

\* Blood smears negative.

observed in fowls 124, 148 and 150, where the titre increased only to 1/512. The greatest response was observed in fowl 126, in which the titre rose from 1/256 to 1/65,536. A further increase in agglutinating titre was observed in every case tested 5 to 7 days after the fifth injection. Fowl 126 reached the highest agglutinating titre, the readings being 1/256 before injection, 1/65,536 after the third injection, and 1/262,144 after the fifth injection. Fowl 125 showed the greatest increase in agglutinating titre, following injections, from 1/16 in the normal fowl to 1/16,384 after the third injection and 1/131,072 after the fifth injection. The least increase in titre was observed in fowl 124, from 1/256 in the normal fowl to 1/512 after three injections, and 1/32,768 after five injections.

These experiments show conclusively that agglutinins are readily produced in fowls by the repeated injection of large numbers of inactivated sporozoites of *P. gallinaceum*. The specificity of the response is indicated by the progressive increase in agglutinating titre after repeated injections. The agglutinating titres of the sera of fowls repeatedly injected with inactivated sporozoites were appreciably higher than those observed in the sera of fowls which had suffered



from acute or chronic infections with the homologous Plasmodium as will be seen from a comparison of Tables III and IV. The highest agglutinating titre of the serum of six chronic *gallinaceum* infections produced in fowls by blood inoculation was 1/16,384 (fowl 12), and of two acute *gallinaceum* infections was only 1/128 (Table IV).

The susceptibility of fowls which received repeated injections of inactivated sporozoites to infections with the homologous Plasmodium will be dealt with in another paper.

#### SUMMARY AND CONCLUSIONS.

(1) A method is described for inactivating sporozoites of *P. gallinaceum* in saline dissections of salivary glands of mosquitoes by exposure to ultra-violet radiation.

(2) Repeated injections of inactivated sporozoites of *P. gallinaceum* into fowls produced a specific agglutinogenic effect.

(3) The agglutinating titre of serum from fowls which received repeated injections of large doses of inactivated sporozoites of *P. gallinaceum* was higher than that observed in the serum of fowls with acute or chronic infections induced by blood inoculation of the homologous Plasmodium.

#### REFERENCE.

- MULLIGAN, H. W., RUSSELL, P. F., Specific agglutination of sporozoites. *J. Mal. Inst. Ind.*, **3**, **4**, pp. 513-524.  
and MOHAN, B. N. (1940).



## ACTIVE IMMUNIZATION OF FOWLS AGAINST *PLASMODIUM GALLINACEUM* BY INJECTIONS OF KILLED HOMOLOGOUS SPOROZOITES\*.

BY

MAJOR H. W. MULLIGAN, I.M.S.

(Director, Pasteur Institute of Southern India, Coonoor),

PAUL F. RUSSELL,

(Field Staff, International Health Division of the Rockefeller Foundation),

AND

BADRI NATH MOHAN

(Senior Technical Assistant, Malaria Investigations).

[February 15, 1941.]

### INTRODUCTION.

MULLIGAN, Russell and Mohan (1940) reported that normal serum and heterologous malarial sera did not agglutinate sporozoites in saline dilutions above 1/128, whereas sera from fowls, sparrows, and human patients, with chronic malarial infections, agglutinated sporozoites of the homologous Plasmodium in saline dilutions ranging from 1/1,000 to 1/65,000. It was concluded that sporozoite agglutination in high dilutions of malarial sera was a specific reaction.

In a subsequent paper, Russell, Mulligan and Mohan (1941) described a method for inactivating sporozoites of *P. gallinaceum*, in saline dissections of salivary glands of mosquitoes, by exposure to ultra-violet radiation. It was observed that repeated injections of inactivated sporozoites of *P. gallinaceum* into normal fowls produced a specific agglutinogenic effect. The agglutination titre of serum from fowls which received repeated injections of large doses of inactivated sporozoites was usually higher than that observed in the serum of

---

\* These studies were made under the auspices, and with the support, of the International Health Division of the Rockefeller Foundation co-operating with the Pasteur Institute of Southern India. The authors are indebted to P. Balarama Menon for technical assistance.



fowls with acute or chronic infections induced by blood inoculation of the homologous *Plasmodium*.

This paper reports a series of observations on the susceptibility of fowls to mosquito-borne infection with *P. gallinaceum*, after repeated injections of inactivated sporozoites had considerably increased the agglutination titre of the fowls' sera to homologous sporozoites.

### MATERIALS AND METHODS.

The materials and methods employed in the experiments to be discussed in this paper were essentially similar to those which have been described previously by the authors (1940; 1941).

The routine procedure for infecting experimental or control fowls with *P. gallinaceum* was to allow one or two infected mosquitoes to feed to repletion after applying them over the breast of the fowls. In all cases, the presence of sporozoites in the glands of the mosquitoes used was confirmed by subsequent dissection.

Sporozoites were inactivated by ultra-violet radiation.

Prepatent (or incubation) periods were reckoned from the day of biting by an infected mosquito to the first day on which parasites were detected in routine daily blood film examinations.

The intensity of infection in both experimental and control fowls was estimated on the basis of parasite prevalence in daily blood films. The degree of parasitæmia present was graded in accordance with the following classification, after estimating the percentage of parasitized erythrocytes:—

- + = not more than 10 per cent of erythrocytes parasitized;
- ++ = more than 10 but not more than 40 per cent of erythrocytes parasitized;
- +++ = more than 40 but not more than 70 per cent of erythrocytes parasitized;
- ++++ = more than 70 per cent of erythrocytes parasitized.

### RESULTS.

#### 1. COURSE OF INFECTIONS WITH *P. GALLINACEUM* IN NORMAL FOWLS.

The results of infection with *P. gallinaceum*, induced by mosquito bite, in normal domestic fowls are shown in Table I. There was considerable variation in the intensity of infections induced in this way. The bite of a single infected mosquito may, but does not always, result in infection. In 18 normal fowls fed on by a single infected mosquito, four failed to develop infection during observation periods extending from 15 to 50 days from the time of biting. In eight normal fowls, on which two infected mosquitoes were fed (simultaneously in seven cases and at an interval of 5 days in one case), no failure to produce infection was observed. In 26 normal fowls, fed on by one or two infected mosquitoes\*, four (15·4 per cent) failed to develop infection, 11 (42·3 per cent) developed infections of moderate intensity from which

---

\* Mosquitoes used in these experiments almost invariably showed very heavy sporozoite infections.



recovery was spontaneous, and 11 (42.3 per cent) developed severe fatal infections.

TABLE I.

*Susceptibility of normal fowls to mosquito-borne infection with P. gallinaceum.*

Serial number of fowl.	Number of infected mosquitoes fed.	Prepatent period in days.	Peak of infection.	RESULT OF INFECTION.		
				Died (D).	Spontaneous recovery (SR).	No infection (—ive).
47	1	..	..	..	..	—ive
59	1	11	+++	..	SR	..
63	1	9	++	..	SR	..
109	1	9	+++	..	..	..
110	1	8	+++	D	..	..
111	1	..	..	..	..	—ive
112	1	10	+++	D	..	..
118	1	..	..	..	..	—ive
122	1	10	+	..	SR	..
131	1	7	+++	D	..	..
140	1	16	+	..	SR	..
141	1	..	..	..	..	—ive
141	1	14	++	D	..	..
142	1	12	+	..	SR	..
143	1	8	++	..	SR	..
144	1	8	+	..	SR	..
145	1	8	+	..	SR	..
147	1	12	+++	D	..	..
61	2	7	+++	D	..	..
117	2	9	+++	D	..	..
134	2	9	++	..	SR	..
146	2*	13*	++	..	SR	..
158	2	10	+++	D	..	..
159	2	10	++	..	SR	..
160	2	19	+++	D	..	..
164	2	9	+++	D	..	..
Total = 26		Average = 10.4 days.		Total = 11 or 42.3 per cent.	Total = 11 or 42.3 per cent.	Total = 4 or 15.4 per cent.

\* Mosquitoes fed at an interval of 5 days.

Prepatent period was 8 days after second mosquito fed.

When three or four infected mosquitoes were fed on normal fowls, infection never failed to develop. Such infections appeared to be somewhat more severe than those occurring in fowls in which infection was produced by the bites of one or two mosquitoes, and the average prepatent period was reduced by as much as 2 days, compared with that seen after the bites of not more than two mosquitoes. Sufficient data were not, however, available to confirm the observation of Christophers (1911) that bites of numerous heavily infected mosquitoes produced more severe infections in sparrows than the bite of a single heavily infected mosquito. No more than two infected mosquitoes were used to infect



fowls which had received repeated injections of inactivated sporozoites, since it was desired to avoid giving an overwhelming infective dose of viable sporozoites.

## 2. COURSE OF INFECTION WITH *P. GALLINACEUM* IN FOWLS WHICH RECEIVED REPEATED INJECTIONS OF INACTIVATED SPOROZOITES.

The results of infection with *P. gallinaceum*, induced by the bites of one or two infected mosquitoes, in 14 fowls which had received repeated injections of inactivated sporozoites, are shown in Table II.

The agglutination titre of the serum of 13 of these fowls was ascertained within 5 to 7 days after the last injection of inactivated sporozoites, and was found to be considerably above the normal limits in every case. Only one of these 14 fowls failed to develop infection induced by mosquito bite. Since this fowl (96) was fed on by only a single infected mosquito, this finding cannot be regarded as abnormal (Table I). The remaining 13 fowls in the series developed infections ranging from mild or moderate attacks, followed by spontaneous recovery, to severe fatal attacks. For the series as a whole, one (7.1 per cent) failed to become infected, nine (64.3 per cent) recovered spontaneously and four (28.6 per cent) developed severe infections and died. Compared with the corresponding figures for normal fowls (Table I), these findings indicate that fowls which had received repeated injections of inactivated sporozoites were appreciably less susceptible to the pathogenic effects of mosquito-borne *P. gallinaceum* infection than normal fowls. This suggests some degree of protection.

When the intensity of infection in experimental fowls is considered in relation to the agglutination titre of the serum of individual fowls, after the last injection of inactivated sporozoites, the evidence for partial protection becomes statistically significant (Table III). In six fowls (80, 81, 82, 79, 121, and 123), in which the maximum agglutination titre of the serum did not exceed 1/16,000, three (50 per cent) developed severe fatal infections, two (33.3 per cent) developed heavy infections (++) from which recovery was spontaneous, and only one (16.7 per cent) escaped with a mild infection (+). In striking contrast to these figures, the six fowls (124, 127, 150, 125, 128 and 126) in which the agglutination titre of the serum was 1/32,000 or more, developed only very mild infections (+) in each case, even when two infected mosquitoes were used (fowls 127, 150). Although these figures are small, evidence of protection in fowls in which the agglutination titre of the serum was at least 1/32,000 is apparent.

It seems fair to conclude, therefore, that repeated injections into fowls of inactivated sporozoites of *P. gallinaceum*, producing an agglutination titre of at least 1/32,000, render such fowls partially immune to the pathogenic effects of mosquito-borne infection with the homologous Plasmodium.

## DISCUSSION OF RESULTS.

The mechanism of defence against malaria has been the subject of numerous papers, somewhat sharply divided into two groups. On the one hand, Taliaferro and his co-workers (Taliaferro, 1931; Cannon and Taliaferro, 1931; Taliaferro and Cannon, 1936; Taliaferro and Mulligan, 1937), and other authors, have demonstrated and emphasized the important part played by the lymphoid-macrophage system in immunity against malaria. On the other hand,



TABLE II.  
Susceptibility of fowls to mosquito-borne infection with *P. gallinaceum* after repeated injections of killed homologous sporozoites.

Serial number of fowl.	INJECTIONS OF KILLED SPOOROZOITES.		Highest observed agglutination titre after completion of injections.	Number of mosquitoes used to produce infection.	Prepatent period in days.	Peak of infection.	RESULT OF INFECTION.		
	Number of injections.	Total number of mosquitoes.					Died (D).	Spontaneous recovery (SR).	No infection (-ive).
79	4	70	1/8192	1	8	+++	D	..	..
80	4	70	1/1024	1	10	+	..	SR	..
81	4	70	1/2048	1	9	+++	D	..	..
82	4	70	1/16384	1	12	+++	..	SR	..
96	3	60	1/8192	1	..	..	..	..	-ive
99	3	60	..	1	10	++++	D	..	..
121	5	190	1/16384	1	11	++++	D	..	..
123	5	200	1/16384	1	15	+++	..	SR	..
124	5	200	1/32768	1	9	+	..	SR	..
125	5	190	1/131072	1	8	+	..	SR	..
126	5	190	1/262144	1	12	+	..	SR	..
127	5	200	1/65536	2*	9	+	..	SR	..
128	5	200	1/131072	1	12	+	..	SR	..
150	5	200	1/32768	2	10	+	..	SR	..
Total = 14					Average = 10.4 days.		Total = 4 or 28.6 per cent.	Total = 9 or 64.3 per cent.	Total = 1 or 7.1 per cent.

\* Mosquitoes fed at an interval of 7 days. Parasites present in peripheral blood 2 days after second mosquito fed.



Coggeshall and Kumm (1937) were the first to demonstrate the existence of protective antibodies in the serum of monkeys with a high degree of acquired immunity to malaria. This work has been amply confirmed by other investigators.

TABLE III.

*Susceptibility of fowls to mosquito-borne infection with P. gallinaceum in relation to agglutination titre of serum.*

Fowl number.	AGGLUTINATION TITRE 1/16384 OR LESS.				Result.
	Titre.	Number of mosquitoes used.	Prepatent period.	Peak of infection.	
79	1/8192	1	8	+++	D
80	1/1024	1	10	+	SR
81	1/2048	1	9	+++	D
82	1/16384	1	12	++	SR
121	1/16384	1	11	++++	D
123	1/16384	1	15	++	SR
AGGLUTINATION TITRE 1/32768 OR HIGHER.					
124	1/32768	1	9	+	SR
125	1/131072	1	8	+	SR
126	1/262144	1	12	+	SR
127	1/65536	2	9	+	SR
128	1/131072	1	12	+	SR
150	1/32768	2	10	+	SR

More recently, Mulligan, Sommerville and Swaminath (1940a; 1940b) have reported observations on monkeys which, for the first time, appear to correlate cellular and humoral factors in immunity to malaria. Their results suggest that cellular and humoral agencies in defence against malaria are so closely interdependent that a full measure of one is relatively ineffective in the absence of an adequate measure of the other.

Earlier attempts to demonstrate the presence of protective antibodies in the serum of birds with a high degree of acquired immunity to malarial infection were attended with negative (Moldovan, 1912; Taliaferro and Taliaferro, 1929; Sergeant and Catanei, 1937) or inconclusive results (Findlay and Brown, 1934; Hegner and Eskridge, 1938; Hegner and Dobler, 1939). More recently, however, the existence of protective antibodies in avian malaria has been clearly demonstrated by the work of Manwell and Goldstein (1938; 1940) and



errio and Taliaferro (1940). In these successful experiments, large doses of immune serum were used, the injections were administered over relatively long periods, and serum treatment was commenced before, or concurrently with, the infecting dose of parasites. In the light of the experiments reported by Mulligan *et al.* (1940a), it seems possible that the early administration of large doses of immune serum may have been instrumental in bringing about a degree of cellular stimulation sufficient to facilitate the utilization of protective antibodies to good advantage. In this connection, it is interesting to note that Manwell and Goldstein (1940) observed considerable enlargement of the spleen in birds which received repeated injections of immune serum.

Mulligan *et al.* (1940c) pointed out that the spleen is of little importance in relation to the high degree of natural resistance possessed by *sinicus* monkeys to infection with *P. cynomolgi*. Herman and Goldfarb (1939) arrived at a similar conclusion in regard to the very low grade infection caused by *P. circumflexum* in chickens. These findings cannot, however, be admitted as evidence against the important part played by the lymphoid-macrophage system in the acquisition of immunity to malarial infections to which the host is moderately or highly susceptible (Mulligan *et al.*, 1940c).

Attempts to produce active immunization of birds and animals against malaria by experimental procedures appear to have been relatively seldom undertaken. In 1933, one of us (H. W. M.) failed to protect *rhesus* monkeys against severe fatal attacks with *P. knowlesi* by previous injections of large numbers of killed homologous parasites (unpublished work). Parasite material was obtained from very heavily infected blood by laking the erythrocytes with distilled water. It was thought at the time that this treatment might have destroyed the antigenic properties of the parasite material. Using somewhat similar technique, Eaton and Coggeshall (1939) also failed to obtain evidence of active immunity. They state that 'in the experiments on active immunization of monkeys, injection of parasites killed by heat, freezing and thawing, formalin, or drying apparently produced no resistance whatever to infection with living parasites'. The authors considered that a possible explanation of their failure was the lability of the antigen by the methods employed. Shortt and Menon (1940) failed to immunize *sinicus* monkeys against *P. knowlesi* by repeated injections of large doses of parasites obtained from heavily infected blood. Parasite material was separated from erythrocytes by *saponin hæmolysis*\*, and was then ground up with carborundum to kill the parasites. The likelihood of the antigenic structure of the parasite having been impaired in the latter experiment would appear to have been greatly reduced. So far, no successful active immunization against malaria appears to have been effected by the injection of blood parasites.

Attempts have also been made to produce active immunization against malaria by the use of sporozoites. Boyd and Kitchen (1936) observed no immunity in human patients on whom mosquitoes with degenerate sporozoites were fed. Sergent and Sergent (1910) attempted to immunize birds against infection with *P. relictum* by injecting sporozoites devitalized *in vitro* by

---

\* Christophers and Fulton (1938) found that parasites separated from erythrocytes in this way were infective.



exposure to low temperatures for 12 to 48 hours. Birds which remain infected after this treatment were as susceptible to subsequent infection by mosquito bite as normal birds.

Failure to produce active immunization against malaria, whether by injection of sporozoites or trophozoites, may have been attributable to a variety of causes, such as (1) the administration of insufficient antigenic material, or (2) partial or complete destruction of the antigenic structure of the parasite material employed.

The experiments reported in this paper appear to afford the first suggestive evidence of the possibility of producing partial immunity against malarial infection as the result of active immunizing procedures. It seems probable that the success of these experiments may have been due to the administration of sufficient quantities of material, the antigenic properties of which were adequately preserved. In this connection, it may be pointed out that exceptionally large quantities of sporozoites were injected over relatively long periods of time, and that some evidence is available that such immunity as was produced was observed only in fowls which received the largest doses of inactivated sporozoites. Furthermore, sporozoites were injected into fowls within half-an-hour of dissection of salivary glands, and received no chemical or other treatment, except ultra-violet radiation.

It is of particular interest that partial protection against mosquito-bite infection was regularly observed only in those fowls in which the agglutinating titre of the serum was at least 1/32,000. This does not necessarily indicate that protection is directly dependent upon the phenomenon of agglutination. Many believe in the identity of precipitins, lysins, agglutinins, etc., and it is possible that the partial protection observed in fowls with a high agglutination titre may have been due to some immune phenomenon other than agglutination. The agglutination titre of the serum, however, may be an index of a high concentration of humoral protective antibodies, which may be necessary before protective action against mosquito-borne infection becomes apparent.

In addition to the evidence favouring the occurrence of a high concentration of protective antibodies in fowls which had received repeated injections of inactivated sporozoites, it must be remembered that the cellular defence mechanism may have been stimulated by repeated injections of saline suspensions of heavily infected gland tissue. So far, no histological evidence of such stimulation has been obtained. Attempts are now being made to stimulate the cellular defence mechanism of fowls in which the agglutinating titre of the serum has been brought to a high level by sporozoite injections, in the hope that, in this way, an even greater degree of active immunity may be demonstrable.

It is not clear from our experiments whether the partial protection afforded to fowls against mosquito-bite infection, by reason of active immunization with killed sporozoites, is effective against sporozoites or trophozoites, or both. So far, no experiments have been carried out to determine what degree of immunity (if any) would be apparent against infections made by blood inoculation (trophozoite infection). The question of the antigenic identity of sporozoites and trophozoites is one of considerable importance. Boyd and Kitchen (*loc. cit.*) and Boyd, Stratman-Thomas and Kitchen (1936) have suggested that acquired antiparasitic immunity observed in certain malarial infections is directed, mainly at least, against trophozoites. Sinton (1940) inclines to the same view, although his experiments with *P. ovale* indicated, as he points out, that possibly



a true immunity against sporozoites could be developed. Wolfson and Causey (1939) conclude, as the result of experiments in birds with two strains of *P. cathemerium*, that immunity to superinfection is probably effective against sporozoites as well as trophozoites.

The actual amount of parasite material (sporozoites) injected into fowls in which some evidence of protection was afterwards observed by us was probably proportionately less than the amount of parasite material (trophozoites) injected into monkeys by Shortt and Menon (*loc. cit.*) without producing any detectable degree of active immunity. It is possible, therefore, that sporozoites are antigenically more potent than trophozoites. Sinton (*loc. cit.*) attributes a greater 'aggressivity' to sporozoites of *P. ovale* than to trophozoites of the same species, and points out that immunity developed as the result of infection induced by sporozoites is more effective than immunity developed as the result of infections induced by blood inoculation (trophozoite infection). Russell *et al.* (*loc. cit.*) have shown that the agglutination titre of fowl serum is often higher after repeated injections of inactivated sporozoites than that of the sera of fowls with acute or chronic infections with the homologous Plasmodium, induced by blood inoculation. It may eventually be found that sporozoites are more potent antigenically than trophozoites.

### SUMMARY AND CONCLUSIONS.

Repeated injections of large numbers of inactivated sporozoites of *P. gallinaceum* into domestic fowls caused a considerable rise in the agglutination titre (against homologous sporozoites) of the serum, in every case. Fowls treated in this way were subsequently found to be susceptible to mosquito-borne infection with the homologous strain of *P. gallinaceum*. Those in which the agglutination titre was 1/16,000 or less appeared to be as susceptible to the pathogenic effects of this infection as normal fowls (mortality 50 per cent), but those in which the agglutination titre was 1/32,000 or higher, developed only very mild infections in every case (mortality nil).

The conclusion is reached that it is possible, by injecting large doses of inactivated sporozoites into fowls, to bring about partial active immunization against mosquito-borne infection with the homologous Plasmodium, when a sufficient degree of antigenic response has been elicited.

### REFERENCES.

- BOYD, M. F., and KITCHEN, S. F. *Amer. J. Trop. Med.*, **16**, 3, pp. 317-322. (1936).  
 BOYD, M. F., STRATMAN-THOMAS, W. K., and KITCHEN, S. F. (1936). *Ibid.*, **2**, pp. 139-145.  
 CANNON, P. R., and TALIAFERRO, W. H. (1931). *J. Prev. Med.*, **5**, 1, pp. 37-64.  
 CHRISTOPHERS, S. R. (1911). *Scientific Memoirs, Govt. of India*. No. 46, pp. 118-125.  
 CHRISTOPHERS, S. R., and FULTON, J. D. (1938). *Ann. Trop. Med. Parasit.*, **32**, 1, p. 59.  
 COGGESHALL, L. T., and KUMM, H. W. (1937). *J. Exp. Med.*, **66**, 2, pp. 177-190.  
 EATON, M. D., and COGGESHALL, L. T. (1939). *Ibid.*, **70**, 2, pp. 141-146.



# 34      *Active Immunization of Fowls against P. gallinaceum.*

- FINDLAY, G. M., and BROWN, H. C. *Brit. J. Exp. Path.*, **15**, p. 148.  
(1934).
- HEGNER, R., and DOHLER, M. (1939). *Amer. J. Hyg.*, **30**, 3, Sec. C., pp. 81-91.
- HEGNER, R., and ESKRIDGE, L. *Ibid.*, **28**, 3, pp. 367-376.  
(1938).
- HERMAN, C. M., and GOLDFARB, A. I. *Amer. J. Trop. Med.*, **10**, 6, pp. 595-598.  
(1939).
- MANWELL, R. D., and GOLDSTEIN, F. *Proc. Exp. Biol. Med.*, **30**, p. 426.  
(1938).
- Idem* (1940) .. *J. Exp. Med.*, **71**, 3, pp. 400-422.
- MOLDOVAN, J. (1912) .. *Centralbl. f. Bakt. Orig.*, **60**, pp. 105-110.
- MULLIGAN, H. W., RUSSELL, P. F., *J. Mal. Inst. Ind.*, **3**, 4, pp. 513-524.  
and MOHAN, B. N. (1940).
- MULLIGAN, H. W., SOMMERVILLE, T., *Ibid.*, **3**, 4, pp. 563-579.  
and SWAMINATH, C. S. (1940a).
- Idem* (1940b) .. *Ibid.*, **3**, 4, pp. 581-590.
- Idem* (1940c) .. *Ibid.*, **3**, 4, pp. 591-601.
- RUSSELL, P. F., MULLIGAN, H. W., *Ibid.*, **4**, 1, pp. 15-24.  
and MOHAN, B. N. (1941).
- SERGENT, ET., and CATANEI, A. (1937). *Arch. Inst., Past. D'Alger.*, **15**, 1, pp. 18-19.
- SERGENT, ET., and SERGENT, EDM. *C. R. Ac. Sciences*, **151**, p. 407. (Cited in *Bull. l'Inst Past.*, 1911, **9**, p. 179.)  
(1910).
- SHORTT, H. E., and MENON, K. P. *J. Mal. Inst. Ind.*, **3**, 1, pp. 191-193.  
(1940).
- SINTON, J. A. (1940) .. *Trans. Roy. Soc. Trop. Med. Hyg.*, **33**, 4, pp. 439-446.
- TALIAFERRO, W. H. (1931) .. *South. Med. J.*, **24**, 5, pp. 409-415.
- TALIAFERRO, W. H., and CANNON, *J. Inf. Dis.*, **59**, 1, pp. 72-125.  
P. R. (1936).
- TALIAFERRO, W. H., and MULLIGAN, *Ind. Med. Res. Mem.*, No. 29, pp. 1-138.  
H. W. (1937).
- TALIAFERRO, W. H., and TALIAFERRO, *J. Prev. Med.*, **3**, pp. 209-223.  
L. G. (1929).
- Idem* (1940) .. *J. Inf. Dis.*, **66**, 2, pp. 153-165.
- WOLFSON, F., and CAUSEY, O. R. *J. Parasit.*, **25**, 6, pp. 510-511.  
(1939).



## EXPERIMENTAL MALARIA CONTROL IN A HYPERENDEMIC TEA GARDEN IN UPPER ASSAM BY THE USE OF PYROCIDE 20 AS AN INSECTICIDAL SPRAY.

BY

D. K. VISWANATHAN, L.M.S., B.S.Sc., M.P.H. (Johns Hopkins)  
(Research Officer, Assam Medical Research Society, Shillong.)

[February 25, 1941.]

### INTRODUCTORY.

Ross (1936) and De Meillon (1936) have reported upon the successful results attending the spray-killing of adult mosquitoes as an antimalarial measure in South Africa. Covell, Mulligan and Afridi (1938) adopted this measure with encouraging results under rural Indian housing conditions, which at first sight 'seemed to offer a forbidding barrier to success'. They were dealing with *A. culicifacies* as the vector species. Russell and Knipe (1939) carried out similar experiments in Pattukkottai, Madras Presidency, and reported good results. They were also dealing with the same vector species. The experiment now recorded was carried out in 1940 in Limbuguri Tea Garden, an hyperendemic area in Upper Assam, where *A. minimus* is the sole vector. In order to arrive at a correct evaluation of the results, only a portion of the garden was sprayed, the rest being left as a comparison area. Two hundred and forty-four coolie houses were included in the sprayed area, out of a total of 599. The houses have mud-plastered katcha brick walls and tin roofs with thatch extensions, and complete closure was neither possible nor attempted. Each house, cattle-shed, outhouse, etc., was numbered, its dimensions measured and a plan and front elevation recorded on a house card. The floor area and cubic content were then calculated and recorded. On the back of the card were recorded the date of spraying, date of catch of adult anophelines, their identification and the results of dissection. From these records it was later possible to collect the data for the experimental and control areas separately.

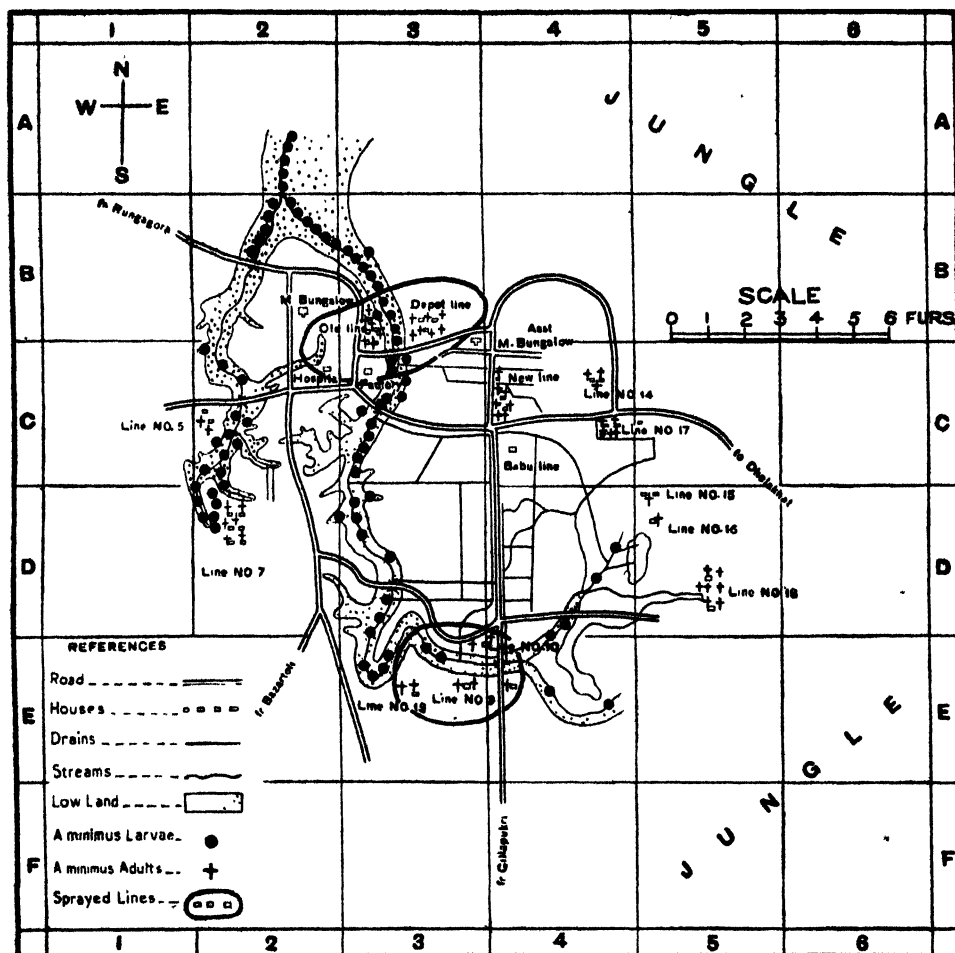
### EPIDEMIOLOGY.

#### 1. LOCATION AND EXTENT.

The garden is situated in Lakhimpur District in Upper Assam, in 27°30' north latitude and 95°20' east longitude, and covers 1,896 acres of which about 614 are under tea cultivation (see Map).



## MAP OF LIMBUGURI TEA ESTATE.

Showing *A. minimus* breeding grounds and adult catch, October 1940.

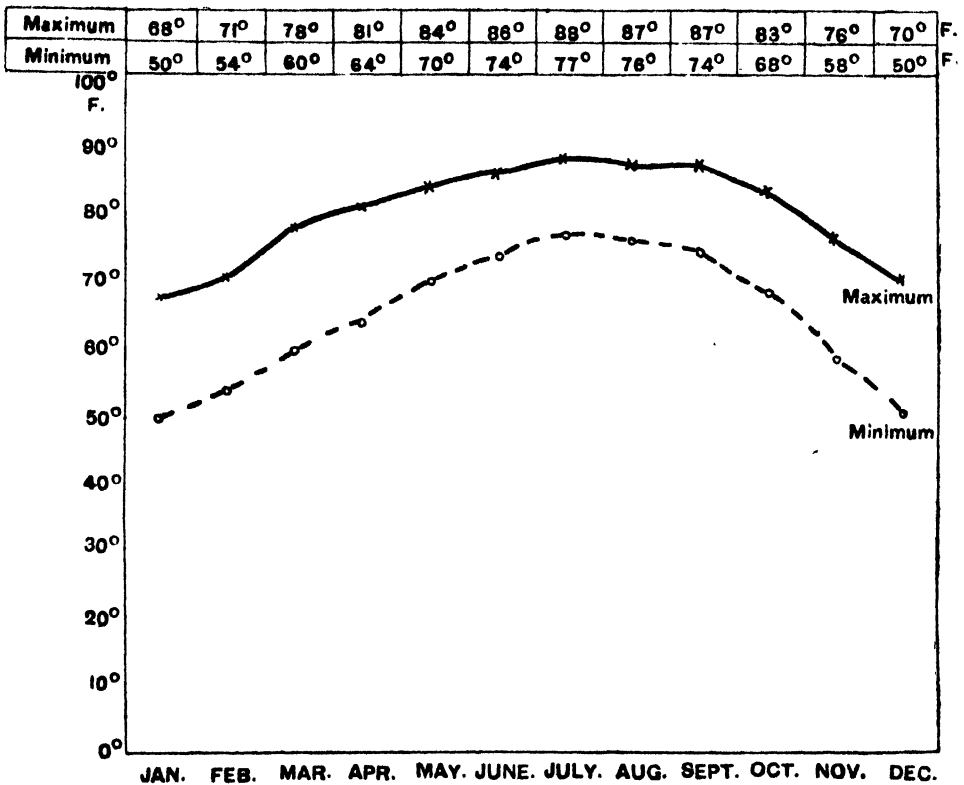
## 2. CLIMATE AND RAINFALL.

There is a well-marked cold season. Towards the end of November, the minimum temperature falls below 60°F. In December, January and February, the daily maximum varies between 67° and 72°F. and the daily minimum between 47° and 56°F. Towards the latter half of March, the daily minimum temperature rises above 60°F. (Graph 1). No humidity records are available. The average annual rainfall during the period 1930 to 1939 was 102.7 inches. Very light showers occur in the first quarter of the year, whilst fitful, but fairly heavy showers fall in April and May in the pre-monsoon period. June, July, August and September register about 68 inches, i.e., two-thirds of the annual rainfall. October marks the tailing off of the monsoon, but there is light occasional rain in November and December (Graph 2).



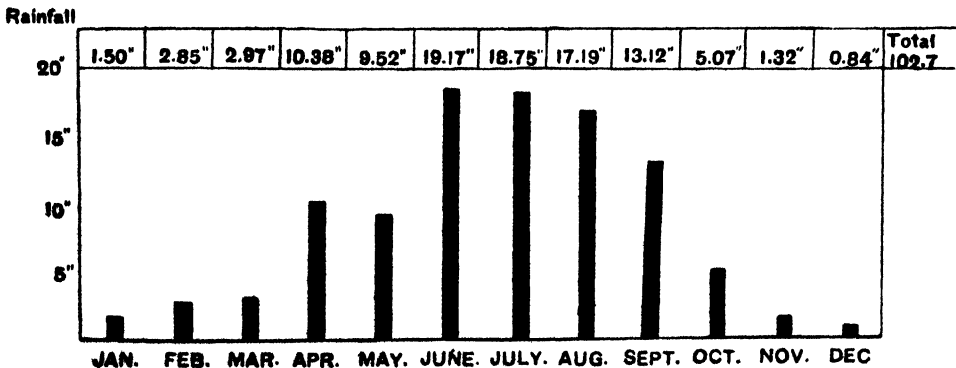
GRAPH 1.

Average daily maximum and minimum temperature by months from 1930 to 1939.



GRAPH 2.

Average monthly rainfall, 1930 to 1939.





## 3. MALARIA INCIDENCE.

Table I shows the annual malaria admissions to hospital from 1933 to 1940, the total cases of all diseases treated every year, the percentage of the former to the latter, the average annual population and the incidence of malaria per mille of population every year.

TABLE I.

*Limbuguri Tea Estate.**Hospital figures—annual malarial incidence.*

	1933.	1934.	1935.	1936.	1937.	1938.	1939.	1940.
Malaria cases ..	4,202	4,151	3,127	1,741	2,353	1,726	1,675	881
All cases ..	12,253	12,693	9,296	8,992	9,025	8,104	8,536	6,864
Percentage of malaria to all cases.	34	33	33	19	26	21	20	13
Average population ..	2,522	2,553	2,764	2,753	2,613	2,624	2,697	2,789
Malaria incidence per mille of population.	1,666	1,626	1,131	632	900	658	621	316

The above figures show that the degree of malarial prevalence is very high in this garden, though the figures since 1936 show a considerable reduction as compared with those of the previous 3 years. A few years ago an attempt was made to deepen the lower reaches of the main *jhan*\*, but the work is incomplete. Quinization of the children has been in vogue and actual fever cases receive adequate treatment. It is difficult to assess how much of the reduction in malaria incidence is attributable to these measures. Whatever be the cause, a downward trend in the malarial incidence per mille of population is evident, and a curve representing this has been depicted in Graph 3. Continuing the curve to 1940, the expected malaria incidence in that year is estimated as 500 per mille of population. The observed incidence was, however, only 316 per mille, a reduction by 37 per cent. The figures, however, relate to the whole garden while the spraying of dwellings was confined to less than half the coolie lines. Besides, spraying was started only in April 1940, and the first 3 months' figures consist mostly of relapses of infections acquired during the previous year.

To afford a more valid comparison, Table II has been compiled showing the number of malaria cases for the period April to December in each of the years 1933 to 1940, together with the incidence per mille of population for that period.

The average malarial incidence in the months of April to December during the period 1933 to 1939 was 922 per mille, whereas it was only 250 per mille in 1940. The reduction is striking. In order to allow for the downward trend due to other causes than the specific one of spraying, which was adopted only in 1940, Graph 4 has been drawn, showing the incidence per mille for the months

\* A term in common use in Assam to indicate a ravine stream.



of April to December from 1933 to 1939, and the curve has been extended to 1940. The expected incidence in 1940 in accordance with this curve is about 480 per mille, while the observed incidence was only 250 per mille, a reduction by nearly 50 per cent. This method of analysis presupposes that the downward

GRAPH 3.

Annual malarial incidence per mille of population, 1933 to 1940.

Malarial incidence  
per mille per annum.

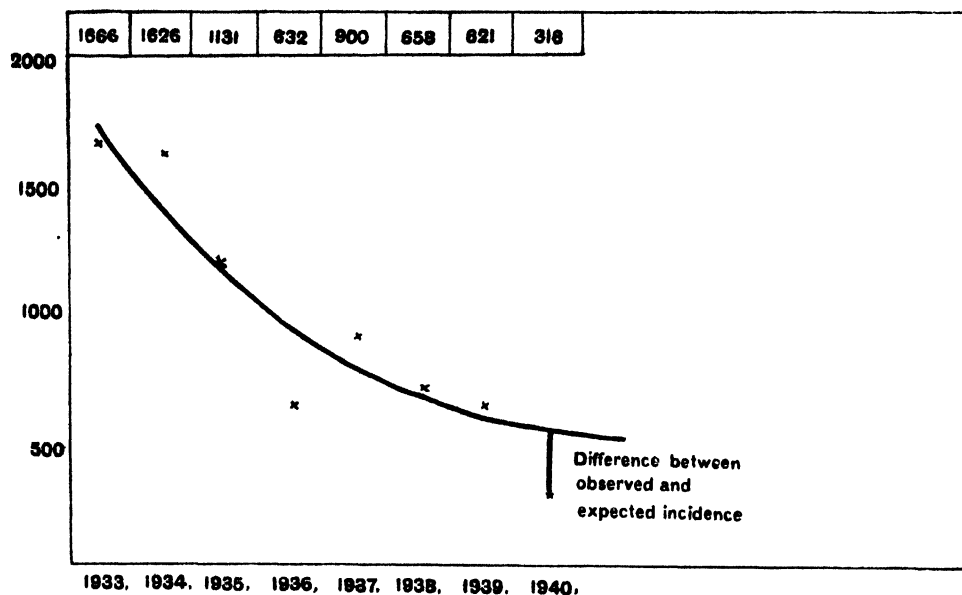


TABLE II.

*Limbuguri Tea Estate.*

*Malarial incidence in the months of April to December from 1933 to 1940.*

	1933.	1934.	1935.	1936.	1937.	1938.	1939.	1940.
Malaria cases ..	3,808	3,758	2,639	1,548	2,150	1,496	1,538	702
Population ..	2,522	2,553	2,764	2,753	2,613	2,624	2,697	2,812
Incidence per mille for the period April to December.	1,506	1,472	955	562	823	570	570	250

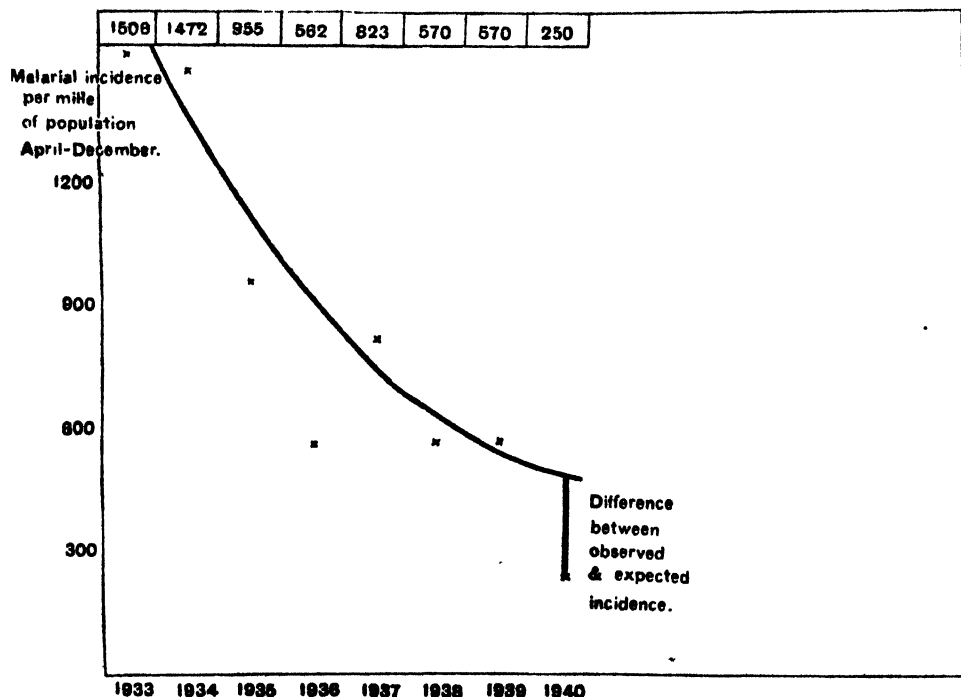
trend is uniform in the period 1933 to 1939 and will be maintained to the same degree later on. Actually, however, the period 1933 to 1939 consists of two parts, i.e., before and after a partial scheme of drainage had been executed. As a result of this drainage, there is a fairly steep drop in 1935-36 and a more



or less uniform level of incidence later. If we took this latter level of incidence as the norm, the reduction in 1940 is even more significant.

GRAPH 4.

Malarial incidence per mille of population in the period April to December, 1933 to 1940.



As only part of the garden was sprayed, one would expect that the malarial incidence in the sprayed area as compared with that in the unsprayed area would furnish a useful guide as to the value of spraying. The relevant figures for 1940 are shown in Table III.

In the first quarter of the year out of 179 cases, 100 occurred in the sprayed group and 79 in the comparison group. If this proportion is taken as indicating the relative prevalence of the disease, the expected number of cases in the sprayed group from April to December is estimated to be 390. Actually, there were 250 cases. Thus, the hospital figures do not show any demonstrably significant variation in the incidence of malaria in the sprayed and unsprayed houses. Coupled with the fact that there is a marked reduction in the malaria incidence in the whole garden, the above finding shows that the effects of even partial spraying are manifest throughout the garden. This may be due to (1) dispersal of mosquitoes taking place so constantly between the sprayed and unsprayed houses as to render partial control manifest throughout the garden. (2) Progressive diminution in the output of vector species, the influence of which is felt throughout the garden, where the main breeding grounds lie between the sprayed and unsprayed portions. The recorded larval and adult catches



TABLE III.

*Limbuguri Tea Estate.**Monthly incidence of malaria among sprayed and unsprayed lines in 1940.*

	January.	February.	March.	April.	May.	June.	July.	August.	September.	October.	November.	December.	Total.
Sprayed	36	36	28	31	26	49	58	55	40	53	42	30	484
Unsprayed	34	26	19	20	24	41	47	48	47	39	29	20	394
TOTAL ..	70	62	47	51	50	90	105	103	87	92	71	50	878

	JANUARY TO MARCH.		APRIL TO DECEMBER.		Total observed.
	Observed.	Expected.	Observed.	Expected.	
Sprayed ..	100	94	384	390	484
Unsprayed ..	79	85	315	309	394
TOTAL ..	179	..	699	..	878

throughout the period of the experiment do not, however, entirely warrant such a hypothesis. (3) The absence of any difference in the two areas may only be apparent rather than real, due to a relatively less number of malarial patients seeking hospital treatment among the residents in the unsprayed areas.

*Seasonal incidence.*—Table IV shows the average monthly malarial incidence from 1933 to 1939 and in 1940 respectively.

TABLE IV.

*Limbuguri Tea Estate.**Average monthly malarial incidence 1933 to 1939 and in 1940.*

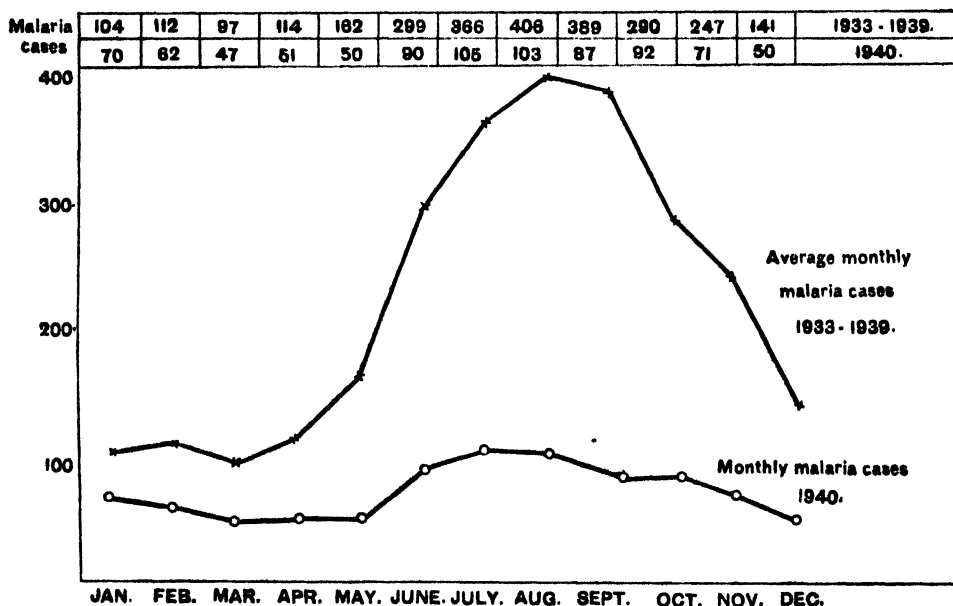
Period.	January.	February.	March.	April.	May.	June.	July.	August.	September.	October.	November.	December.
1933 to 1939	104	112	97	114	162	299	366	406	389	290	247	141
1940 ..	70	62	47	51	50	90	105	103	87	92	71	50



The average incidence during the period 1933 to 1939 shows that March is the least unhealthy month. The curve of incidence rises slowly in April and May and more steeply in June and July, reaches its maximum in August, maintains it in September, and falls slowly in October and November and more steeply in December. In some years, there is a notch in September and a secondary rise in October. June to November seems to be the period of active and effective transmission, which in the earlier stages is apparently facilitated by the spring relapses producing a larger human reservoir of infection. Graph 5

GRAPH 5.

Seasonal malarial prevalence, 1933 to 1939 and 1940.



represents the data compiled in Table IV, and shows that in 1940 the seasonal incidence curve is much flatter. If we reckon the seasonal epidemic index as the ratio of highest monthly morbidity to the lowest, it is 406 : 97, or 4 : 1, in the period 1933 to 1939 and only 105 : 47, or 2 : 1 in 1940. A flattening of the seasonal rise is a fairly sensitive test of the efficacy of any control measure, and the present experiment judged by that test may be deemed as having yielded satisfactory results.

#### 4. RESULTS OF ADULT MOSQUITO CATCHES AND DISSECTIONS.

Approximately 25 man-hours were spent each month from January to December in collecting adult mosquitoes, about half the time in sprayed houses and half the time in unsprayed houses. Table V shows the results of such catch.

Out of 4,022 adult anophelines caught in the year, 1,717, or 42·7 per cent, were *A. minimus*, the most prevalent species. *A. vagus* accounted for 35·6 per



TABLE V.  
*Limbuguri Tea Estate.*  
*Adult anopheline catch.*

	January.	February.	March.	April.	May.	June.	July.	August.	September.	October.	November.	December.	Total.
<i>A. minimus</i> ..	10	3	1	2	6	67	263	193	335	458	272	107	1,717
<i>A. annularis</i> ..	106	71	26	45	94	135	80	89	10	7	44	30	737
<i>A. vagus</i> ..	1	..	..	..	4	40	241	462	604	35	28	17	1,432
Total all species	125	76	27	48	105	244	586	746	949	537	388	191	4,022

cent and *A. annularis* for 18·3 per cent. Six other species (*A. hyrcanus*, *A. culicifacies*, *A. aconitus*, *A. maculatus*, *A. barbirostris* and *A. kochi*) were met with in small numbers, and all of them put together accounted for only 3·4 per cent of the total catch. The seasonal prevalence of the three more common species is shown in Graph 6. It will be noticed that the adult infestation was fairly high in July, August and September, notwithstanding the fact that spraying was carried out from April onwards. There are no similar data for previous years for purposes of comparison, but the current year's data do not indicate any fall in adult anopheline infestation as a result of continued spraying. The degree of *A. minimus* infestation in sprayed and unsprayed areas is shown in Table VI.

TABLE VI.  
*Limbuguri Tea Estate.*  
*Prevalence of A. minimus in sprayed and unsprayed areas.*

Sprayed areas	554	Unsprayed areas	1,163
Per house ..	2·27	Per house ..	3·28
Per capita ..	0·58	Per capita ..	0·63

Entire area .. 1,717 or 2·86 per house  
or 0·61 per capita.

The seasonal prevalence of *A. minimus* in the sprayed and unsprayed areas is shown in Table VII and Graph 7.

In estimating the density of adult anopheline infestation, it is difficult to prescribe standards. If the time factor alone is considered, there is a much higher density in the unsprayed area. If, however, allowance is made for the hypothesis that the greater number of houses and larger population in the unsprayed area would bring about a greater density of adult infestation, even



GRAPH 6.  
Seasonal prevalence of the principal anophelines (adults), 1940.

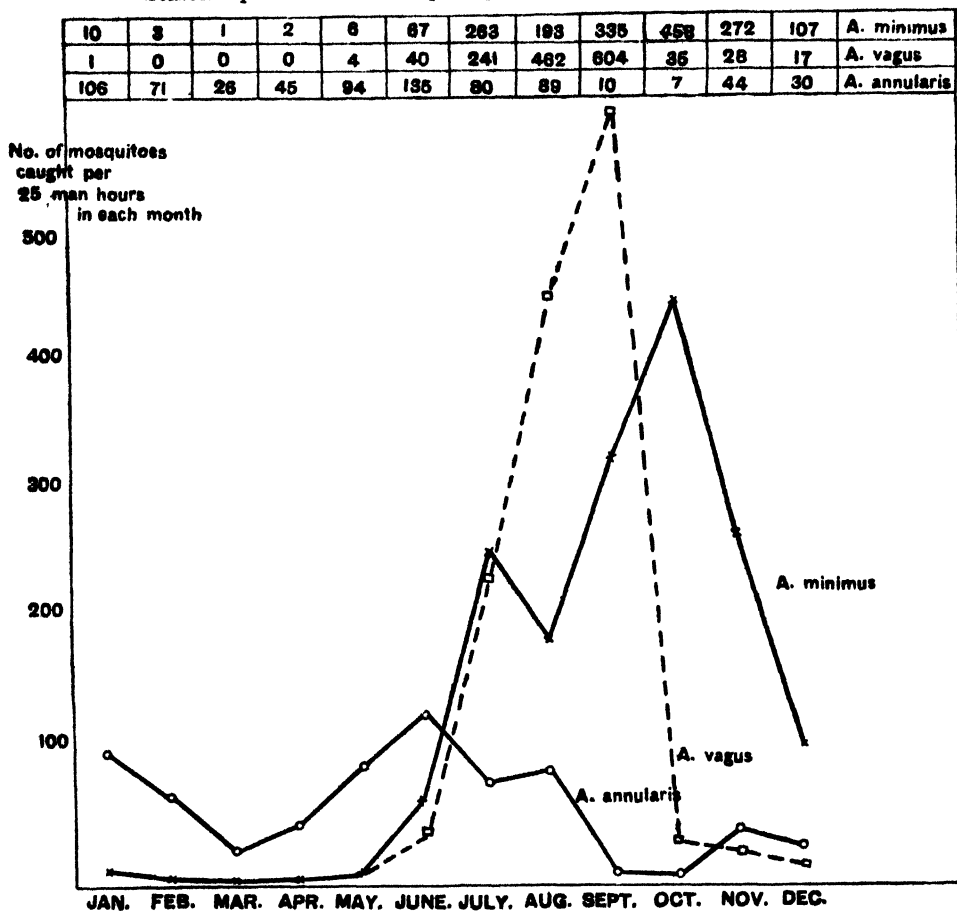


TABLE VII.

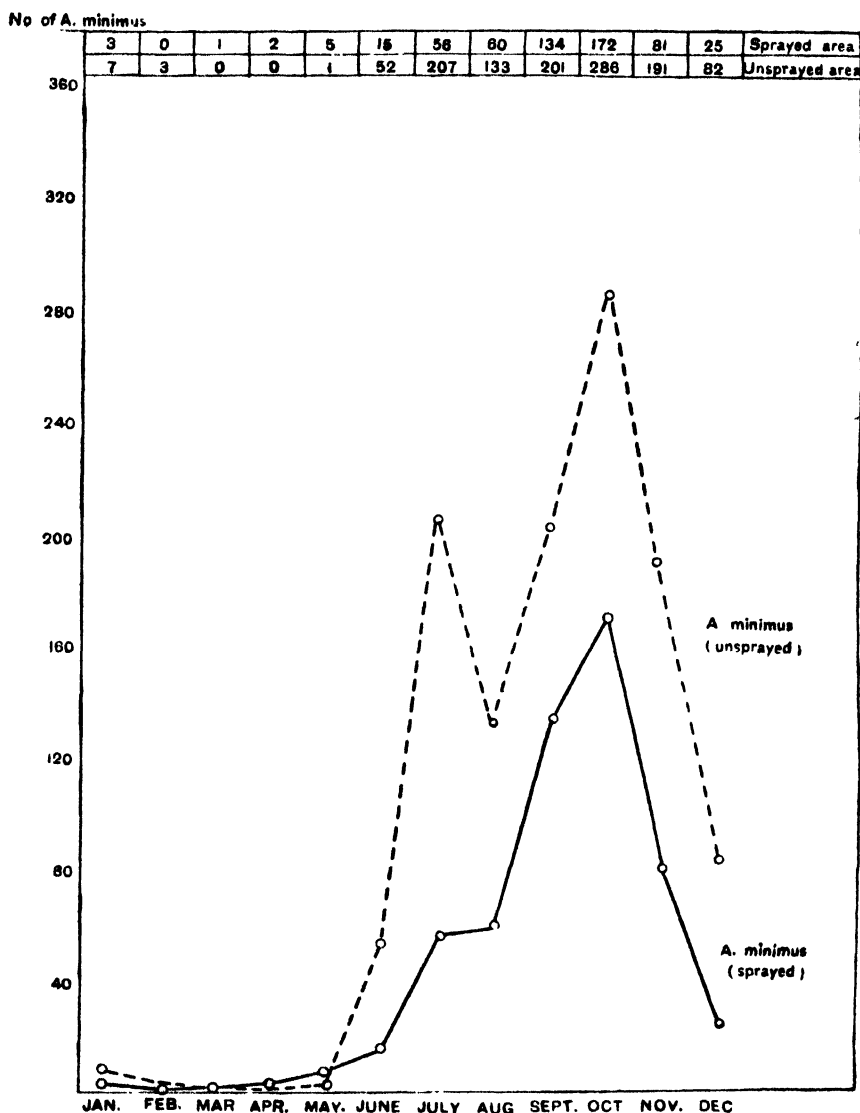
*Limbuguri Tea Estate.*

Seasonal prevalence of *A. minimus* (adults) in sprayed and unsprayed areas, 1940.

	January.	February.	March.	April.	May.	June.	July.	August.	September.	October.	November.	December.	Total.
Sprayed areas ..	3	..	1	2	5	15	56	60	134	172	81	25	554
Unsprayed areas ..	7	3	..	..	1	52	207	133	201	286	191	82	1,163
TOTAL ..	10	3	1	2	6	67	263	193	335	458	272	107	1,717



GRAPH 7.

Seasonal prevalence of *A. minimus* (adults) in sprayed and unsprayed areas, 1940.

then it is found that there is a relatively greater density in the unsprayed area during the period of the experiment.

The most striking variation is, however, in the results of dissections. Out of 421 *A. minimus* dissected in the sprayed area, only 2 gland infections were met with, 1 in June and the other in November. In the unsprayed area, out



TABLE VIII.

*Limbuguri Tea Estate.**Larval findings.*

Species.	JANUARY.		FEBRUARY.		MARCH.		APRIL.		MAY.		JUNE.		JULY.		AUGUST.		SEPTEMBER.		OCTOBER.		NOVEMBER.		DECEMBER.	
	Act.	10 M-H.	Act.	10 M-H.	Act.	10 M-H.	Act.	10 M-H.	Act.	10 M-H.	Act.	10 M-H.	Act.	10 M-H.	Act.	10 M-H.	Act.	10 M-H.	Act.	10 M-H.	Act.	10 M-H.	Act.	10 M-H.
<i>A. minimus</i>	12	4	1	..	..	..	..	..	89	21	79	18	237	47	38	8	194	39	388	84	126	29	81	27
<i>A. aconitus</i>	..	98	7	3	1	..	4	1	41	10	31	8	2	..	5	1	22	4	140	30	385	89	239	79
<i>A. hyrcanus</i>	..	122	260	108	268	96	200	50	101	25	310	78	611	122	607	121	446	89	210	46	246	57	128	42
<i>A. annularis</i>	..	425	48	20	13	5	40	10	92	23	88	22	429	86	359	72	481	96	462	100	730	169	350	116
<i>A. philippinensis</i>	22	7	23	10	..	..	..	..	..	..	1	..	2	..	8	2	8	2	14	3	94	21	43	11
<i>A. barbivostis</i>	..	..	5	2	7	2	2	..	..	..	12	3	12	2	16	3	..	..	..	..	4	1	..	..
<i>A. maculatus</i>	..	..	..	..	2	1	..	..	..	..	..	..	..	..	..	..	..	..	6	1	..	..	..	..
<i>A. pallidus</i>	..	..	1	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..
<i>A. vagus</i>	..	..	..	..	..	..	..	..	51	13	153	38	71	14	57	11	58	12	8	2	1	..	..	..
<i>A. culici/acies</i>	..	..	..	..	..	..	..	..	9	9	2	..	2	..	..	..	..	..	..	..	..	..	..	..
<i>A. subpictus</i>	..	..	..	..	..	..	..	..	..	..	2	..	..	..	..	..	..	..	..	..	..	..	..	..

Act. = Actual number of larvæ collected.

10 M-H = The same reduced per 10 man-hours.



of 705 *A. minimus* dissected, 3 gut infections and 10 gland infections were encountered, 3 each in June, July and August, 2 in September, and 1 each in November and December. The infection rate is, thus, 0.05 per cent in the sprayed area and 1.84 per cent in the unsprayed area, a significant difference.

#### 5. LARVAL SURVEY.

Table VIII records the larval findings in 1940.

Out of a total of 10,202 larvæ caught in 1940, 1,515, or nearly 15 per cent, were *A. minimus*. *A. hyrcanus* and *A. annularis* were the most prevalent species, *A. minimus* third in order and *A. aconitus* and *A. vagus* fourth and fifth respectively.

There are no figures relating to the seasonal intensity of breeding of the various species of anophelines in this area to compare with those compiled during this experiment. The present figures, however, do not show that continued spraying operations bring about a progressive diminution in the number of larvæ.

#### 6. INFANT MALARIA INDEX.

The blood of infants under one year of age was examined every quarter and records kept for individual coolie lines. Table IX shows the infant indices in the sprayed and unsprayed lines respectively in the months of April, June, September, November and December.

TABLE IX.

*Limbuguri Tea Estate.*

*Infant malaria indices in sprayed and unsprayed lines.*

	APRIL.		JUNE.		SEPTEMBER.		NOVEMBER.		DECEMBER.	
	Number examined.	Infant index.	Number examined.	Infant index.	Number examined.	Infant index.	Number examined.	Infant index.	Number examined.	Infant index.
Sprayed ..	29	37.9	27	22.2	28	14.2	26	19.2	27	22.2
Unsprayed ..	48	18.7	51	17.6	57	28.1	55	40.0	52	36.5
TOTAL ..	77	25.9	78	19.2	85	23.5	81	33.3	79	31.6

*Note.*—Infant malaria index = Percentage of infants in whose blood malaria parasites were found.

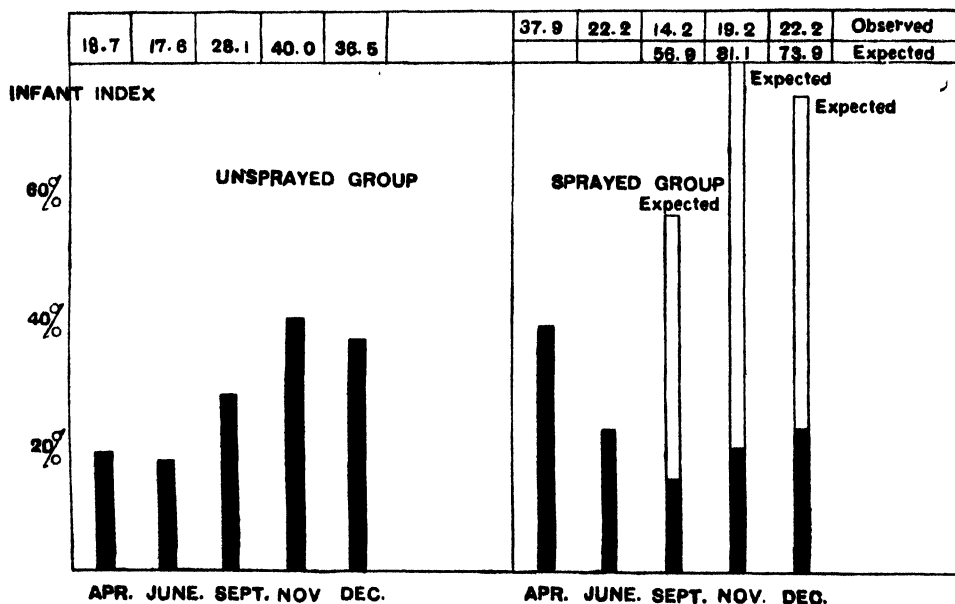
In April, prior to the commencement of spraying, the infant malaria index in the sprayed group was nearly twice as high as that in the unsprayed group. In September and November, the index in the sprayed group was only half that in the unsprayed group, and in December it was about three-fifths of that in the unsprayed group. The absolute reduction in the infant index in the sprayed group amounts to 60 per cent from April to September and about 40 per cent



from April to December. In the unsprayed group, the index has risen by 50 per cent from April to September and by 95 per cent from April to December. Assuming that the infant index in the unsprayed group truly reflects the seasonal hazard, the expected infant index in the sprayed group is 56.9 per cent in September and 73.9 per cent in December. But the observed indices were only 14.2 per cent and 22.2 per cent respectively, showing a relative reduction by 75 per cent in September and 70 per cent in December. This is illustrated in Graph 8.

GRAPH 8.

Infant indices in sprayed and unsprayed lines.



## 7. SPLEEN RATES.

These were compiled thrice during the year, i.e., in January, June and December. Table X shows the number of children between 2 and 10 years of

TABLE X.

	JANUARY.		JUNE.		DECEMBER.	
	Number examined.	Spleen rate.	Number examined.	Spleen rate.	Number examined.	Spleen rate.
Sprayed ..	241	80.5	208	81.2	224	81.6
Unsprayed ..	306	79.7	311	82.6	309	82.2
TOTAL ..	547	80.1	519	82.1	533	81.9



age examined and the spleen rates recorded among them during each of these periods in the sprayed and unsprayed lines respectively.

The spleen rates do not show any significant variation between the sprayed and unsprayed groups in any of the periods under examination.

### 8. PARASITE INDICES.

(a) Table XI shows the parasite indices for the same age group of children in the sprayed and unsprayed lines respectively.

TABLE XI.  
*Limbuguri Tea Estate.*  
*Parasite indices—children 2 to 10 years.*

	JANUARY.		JUNE.		DECEMBER.	
	Number examined.	Parasite index.	Number examined.	Parasite index.	Number examined.	Parasite index.
Sprayed ..	241	50.6	208	38.4	224	27.2
Unsprayed ..	306	43.1	311	43.4	309	42.0
TOTAL ..	547	46.4	519	41.4	533	25.8

While the parasite index among the children from 2 to 10 years of age remained almost at the same level in the unsprayed group in January and December it fell in the sprayed group by about 42 per cent.

(b) Table XII shows the parasite indices among adults.

TABLE XII.  
*Limbuguri Tea Estate.*  
*Parasite indices—adults.*

	JANUARY.		JUNE.		DECEMBER.	
	Number examined.	Parasite index.	Number examined.	Parasite index.	Number examined.	Parasite index.
Sprayed ..	180	13.3	209	9.0	199	4.9
Unsprayed ..	192	9.4	186	12.9	177	12.4
TOTAL ..	372	11.2	395	10.6	376	8.2

The adult parasite index has thus dropped from 13.3 per cent in January to 4.9 per cent in December in the sprayed group, while it has risen from 9.4 per cent in January to 12.4 per cent in the unsprayed group.

### DETAILS OF SPRAYING TECHNIQUE.

Two coolies were employed and they worked from 5 to 8 a.m. It was not possible to spray the houses later than 8 a.m. since the coolies, both men and



women, go out for work by then and frequently lock their doors. About 15 to 20 houses were sprayed per hour, or about 50 houses per day if fairly contiguous. Where the houses were more discrete, only about 30 could be sprayed in 3 hours. The Jim Dandy sprayer and the sprayer made by the Central Technical Works, Lahore, under the guidance of the Director, Malaria Institute of India, were tried. The latter pattern was found more useful in the long run. The coolie walked briskly along the verandah and sprayed under the eaves all round, after which he entered the house and completed the operation. No special precautions were taken to keep the door closed for any specified time after spraying.

The total floor area of the 244 houses sprayed was 104,265 sq. ft., and the total cubic content was 696,700 c. ft. The average floor area per house was 427 sq. ft. and the average cubic content 2,855 c. ft.

One gallon of pyroicide 20 was mixed with 19 gallons of Victoria Brand kerosene and the requirements of one month were generally mixed in advance. Attempts were made to assess the culicidal properties of the mixture at intervals of (a) 1 day after preparation, (b) 1 week later, (c) 2 weeks later, (d) 4 weeks later, and (e) 6 weeks later. A known number of mosquitoes was liberated into a room, and after spraying a measured quantity the dead mosquitoes were recovered and counted. No loss in the culicidal property was

TABLE XIII.  
*Limbuguri Tea Estate.*  
*Statement of quantities of culicide used.*

Month.	QUANTITY OF MIXTURE.			Floor area, sq. ft.	Cubic content, c. ft.
	Gal.	Pts.	Oz.		
April .. ..	9	5	4	352,131	2,369,583
May .. ..	13	1	10	457,463	3,046,674
June .. ..	13	0	11	434,044	2,894,693
July .. ..	13	5	18	461,211	3,098,005
August .. ..	14	2	18	474,128	3,152,627
September .. ..	13	0	16	440,558	2,962,882
October .. ..	14	0	12	460,366	3,068,644
November .. ..	13	5	6	454,353	3,024,422
December (till 15th) ..	6	2	2	208,530	1,393,400
TOTAL ..	111	0	17	3,742,784	25,010,930

noticed till about a month after preparation. When kept longer than this the mixture changed colour and some fall in its culicidal potency was noticed.

Table XIII shows the monthly expenditure of pyroicide 20 and kerosene mixture, and the floor area and cubical content sprayed every month.



In round figures 1 gallon of the mixture would on the average suffice for about 225,300 c. ft., and for the average type of tea garden coolie lines about 33,700 sq. ft., 1 oz. of the mixture will be sufficient for about 1,400 c. ft. or about 200 sq. ft. of floor space.

The total cost of spraying during the period of 8 months and 1 week, viz., from April 7 to December 14, is as follows :—

	Rs.
Pyroicide 20 including freight : 5.56 gallons at Rs. 43 per gallon ..	239
Kerosene including freight : 105.64 gallons at Re. 0-11-3 per gallon ..	75
Wages of 2 coolies at Re. 0-6-0 per day ..	161
Cost of 4 sprayers, including freight charges, etc. ..	30
Miscellaneous, bucket, drum, etc. ..	5
	<hr/> 510

As the total floor area sprayed is 3,742,784 sq. ft. and cubic content sprayed is 25,010,930 c. ft., cost of spraying about 1,400 sq. ft. or about 10,000 c. ft. comes to Re. 0-3-3 1/10. The population in the sprayed group is about 950. The cost per capita works out as Re. 0-8-7 for spraying for the entire malarial season of a little over 8 months.

### DISCUSSION.

In biological studies in the field, it is seldom possible to secure an experimental and comparison area under exactly similar conditions. In the experiment here recorded, a small geographical unit was arbitrarily divided into two parts and the experiment carried out in one leaving the other as a comparison. As can be seen from the map, the main breeding grounds lie almost equidistant from the experimental and comparison areas, and within the effective range of flight of the insect vector. The results are, therefore, strictly comparable.

The malarial morbidity in the experimental and comparison areas did not show any significant difference, although the total malarial incidence showed a considerable reduction at the end of the experiment, even after making allowance for the general downward trend during the last few years. Unfortunately, there are no data for the relative liability of the two areas to the incidence of malaria in the past. The spleen rates in the two areas did not show any significant difference at the beginning of the experiment. While this indicates an equal liability to the incidence of the disease, it throws no light on the relative intensity of infections and, what is more important, on the relative 'hospital-consciousness' of the coolies living in the two areas. The spleen rates are so high in both areas that they cannot show further shades of difference. The fact that the area of experiment was selected on the advice of the manager of the garden suggests that the malarial incidence in that area has usually been higher than elsewhere. Furthermore, at the beginning of the experiment the parasite indices in adults, in children between 2 and 10 years and in infants were all higher in the experimental than in the comparison area. Hence it would seem as though the failure of the hospital figures to demonstrate a lowered morbidity in the experimental area is more apparent than real. Other epidemiological data lend support to this view.



If these other data were not available, one might conclude that the control measures instituted in a portion of the garden benefited the entire estate in the same degree. Such a result would seem logical in an insect-borne disease and would be brought about by two factors, viz., (1) the constant dispersal of the insect and (2) a diminution in the breeding of the insect due to a progressive, though partial, destruction of adults, especially as the breeding grounds lie equidistant from both areas. As regards (2), the recorded data do not show any progressive diminution either in the larval stage or in the adult stage in either of the two areas. As regards (1), the activity of the engorged *A. minimus* is comparatively sluggish. While its flight to the breeding grounds for purpose of oviposition is doubtless necessitated by the instinct of 'species preservation', in between such flights the insect is predominantly a house-rester, and in catching the adult insect one frequently finds that, when disturbed, the insect hardly moves more than a few inches and comes to rest quickly. Such observations, which are frequently made, are inconsistent with its active dispersal. Other epidemiological data to be presently discussed clearly show a very significant reduction in the transmission of malaria in the experimental area. It is, therefore, concluded that the dispersal factor is of little importance.

The most sensitive index of the extent of transmission in a particular season is the infant malaria index, which is almost entirely unaffected by the relapse factor. The infant index in the sprayed area shows a considerable reduction, while in the comparison area it shows a rise. This single datum is alone sufficient to show the successful results of the experiment.

The parasite indices in children from 2 to 10 years of age and in adults also show a similar change, though to a less extent than the infant index, as they are naturally subject to the relapse factor.

The spleen rates show no reduction in the experimental area. Russell and Knipe (*loc. cit.*) record a considerable reduction in spleen rates after spraying for only one season. But they were dealing with 'labile' spleens in an area where malarial endemicity was of very recent introduction (within 5 years) as a result of new irrigation. Such spleens, as they have remarked, 'increase in incidence and size during each malaria season with an ebbing tide during the off season'. Schüffner (1938) has drawn attention to a very important fact in epidemiological studies on malaria, viz., that if the density of infection is considerably higher than is required to maintain the spleen rate at 100 per cent, antimalaria measures may be very effective in reducing the density of infection, but they will fail to make any impression on the spleen rate. He has also stressed the greater amenability of seasonal and freshly introduced malaria to control measures than static and long standing malaria. One cannot, therefore, expect any material reduction in spleen rates in the experimental area, where malaria is hyperendemic, of long standing and has a prolonged period of transmission, as the result of a single year's control measures.

There are no previous records regarding the density of breeding of *A. minimus* or of adult infestation in houses to compare with the data compiled during the experiment. As the breeding grounds lie between the experimental and comparison areas, it is not possible to assess the effects of spraying on the extent of breeding in the absence of any previous data. As regards adult infestation, if the time factor and personal factor are alone taken into consideration, there appears to be some reduction in the population of *A. minimus* in the sprayed area. But spraying once a week has not brought about any spectacular



reduction in the insect population. Such a finding is in accordance with those of other workers. But the most significant difference is in the infection rate of the vector in the two areas. The sprayed areas showed an infection rate of only 0.05 per cent, while in the unsprayed area it was 1.84 per cent. De Meillon (*loc. cit.*) and Covell, Mulligan and Afridi (*loc. cit.*) have drawn attention to the lowered infection rates brought about by spray-killing adult mosquitoes, but the difference noted in the present experiment is of special value in that the experimental and comparison areas were so close to each other. Such a finding shows that, whilst spraying once a week may not bring about a material reduction in the insect population, it certainly affects its life-span.

The results of the experiment show that a given section of a malarious area reacts to local spray-killing of adult mosquitoes when we are dealing with *A. minimus* as a vector. This is of special importance to tea gardens. Antilarval measures require the co-operation of neighbouring interests, since mosquitoes are no respecters of geographical barriers. This measure may then be the method of choice where such co-operation is lacking. Again it may be usefully adopted in the lines in the peripheral portions of the garden, where antilarval measures for half a mile in every direction may be either impracticable or too costly. It may then be adopted as a supplement to antilarval measures within the garden, undertaken to benefit the centrally located lines. It is also of use in some gardens which have deep inaccessible *jhans* where antilarval measure may not be feasible even if funds could be made available. Yet again, some gardens have a small acreage under cultivation and a small labour force, but the breeding grounds of *A. minimus* may be large in extent. Spray-killing of adult mosquitoes will then prove to be far more economical than antilarval measures. One other feature of interest may be recorded here, though it was noted in a rural area and not in the garden under experiment. The people of that area voluntarily brought to the notice of the author the remarkable freedom of their children from eye-sore in June, July and August 1940 after the use of the insecticidal spray. Rural folk are not malaria-conscious, and hence were not in a position to offer any useful information regarding the effect of the control measure on the incidence of malaria. But the absence of eye-sore, which generally occurs in epidemic form in June, July and August, was to them a matter of easy observation and it naturally drew their particular attention. As conjunctivitis in children is generally transmitted from eye to eye during this season by 'mango-flies' or 'eye-flies' as they are variously called, their destruction by the spray presumably brought about an almost total absence of the disease. Such was not, however, the case in the tea garden, in which the hospital figures showed no change in the incidence of cases of eye-sore. The thatched roofing offered the best shelter for adult flies in the rural area and the spraying killed them. In the tea garden it appears that the flies find other suitable places of shelter.

#### SUMMARY OF RESULTS AND CONCLUSIONS.

(1) Weekly spraying with a mixture of pyrocyde 20 and kerosene has brought about a definite reduction in the incidence of malaria in a hyperendemic tea garden.

(2) The malarial incidence in the period April to December was only 250 per mille in 1940 when spraying was in operation, as compared with 570 in 1939, and as against an estimated incidence of 480 per mille in 1940.



(3) There was no material difference in the malaria incidence as shown by the hospital figures among the residents in the sprayed and unsprayed houses. This is attributed in a very small part only to dispersal of mosquitoes. It is possible that the residents in the unsprayed area resort to the hospital less readily for treatment.

(4) The infant malaria index in the sprayed group dropped by 60 per cent from April to September and 40 per cent from April to December, while in the unsprayed group it rose by 50 per cent from April to September and 95 per cent from April to December. The relative reduction in the sprayed group is estimated as about 70 to 75 per cent.

(5) The spleen rates did not show any material change as a result of spraying.

(6) The parasite indices both in the age group 2 to 10, and in adults, showed a definite decline in June and December as compared with that in January in the sprayed group, while in the other group the June and December figures showed a rise over those of January both in children from 2 to 10 years and adults.

(7) Although there are no figures to indicate the seasonal and specific prevalence of anophelines in the past, the data compiled during the experiment do not indicate any progressive diminution in the number of larva produced. There is a slightly higher degree of adult infestation in the unsprayed areas, but this is not very marked.

(8) A very significant difference is noted in the infection rates in adult mosquitoes in the sprayed and unsprayed areas. In the former, only two infections out of 421 *A. minimus* dissected were met with. In the latter 13 infections were encountered out of 705 of the species.

(9) For a population of about 950 living in 250 houses with a floor area of about 100,000 sq. ft. and cubic content of 700,000 c. ft. the cost of weekly spraying comes to about Rs. 510 for the whole season (a little over 8 months). This works at Re. 0-3-3 per 1,400 sq. ft. or 10,000 c. ft. or Re. 0-8-7 per head.

(10) This measure seems especially suitable for tea gardens where the malariogenic breeding grounds consist of inaccessible *jhans*, where co-operative antilarval effort from a neighbouring garden containing breeding grounds within the effective range of flight of vector species of anophelines is lacking, and for controlling malaria on the peripheral portions of a garden while the central portions are more economically dealt with by antilarval methods, and also for gardens with small labour force and relatively extensive breeding grounds.

#### ACKNOWLEDGMENTS.

The author is deeply grateful to Lieut.-Colonel L. A. P. Anderson, I.M.S., Honorary Secretary of the Assam Medical Research Society, for his kind permission to carry out this experiment and for his unstinting advice and guidance during its progress. His thanks are due to Mr. Grayburn, the Manager of the garden, and to Dr. F. C. McCombie, the Medical Officer, for all facilities rendered. Dr. P. C. Bhattacharjee, the Society's Medical Assistant, Babu Nripendranath Bhattacharjee and Babu Surjya Kanta Biswas, the Society's Malaria Inspectors, who were stationed in the garden throughout the period of the experiment, spared no pains in carrying out the duties assigned to them.



## REFERENCES.

- COVELL, G., MULLIGAN, H. W., and AFRIDI, M. K. (1938). An attempt to control malaria by the destruction of adult mosquitoes with insecticidal sprays. *J. Mal. Inst. Ind.*, **1**, 1, pp. 105-113.
- DE MEILLON, B. (1936) .. The control of malaria in South Africa by measures directed against the adult mosquitoes in habitations. *Qlly. Bull. Hlth. Orgn., L. of N.*, **5**, 1, pp. 134-137.
- ROSS, G. A. P. (1936) .. Insecticide as a major measure in control of malaria, being an account of the methods and organization put in force in Natal Zululand during the past six years. *Ibid.*, pp. 114-133.
- RUSSELL, P. F., and KNIPE, F. W. (1939). Malaria control by spray-killing adult mosquitoes. First season's results. *J. Mal. Inst. Ind.*, **2**, 3, pp. 229-236.
- SCHUFFNER, W. A. P. (1938) .. Two subjects relating to the epidemiology of malaria. *Ibid.*, **1**, 3, pp. 221-256.







## OBSERVATIONS ON THE ADULT HABITS OF *ANOPHELES FLUVIATILIS* AND *ANOPHELES VARUNA*.

BY

R. SENIOR WHITE, F.R.S.E.

(*Malariologist, B.-N. Railway, Calcutta*).

(*Inquiry under the Indian Research Fund Association.*)

[February 26, 1941.]

THE completion of the work which forms the subject of this paper has been indefinitely postponed owing to the departure of the author on military duty, but a summary of the available data is here presented for the benefit of other workers who may wish to continue the investigation. Observations were carried out in two areas, the Satpura Ranges in the Central Provinces and the neighbourhood of Vizagapatam in Madras.

### THE SATPURA RANGES.

In the Satpuras, a house for a special collector on the staff of the Indian Research Fund Association was built adjacent to an isolated track-maintenance gang hut at mile 583 of the Calcutta-Bombay main line of the Bengal-Nagpur Railway, in the forests of Khairagarh State on the crossing of the Maikal Range. This hut is approximately 5 furlongs from the nearest village. There are 12 rooms with a population of 11 only at the site and its isolation is such that it is probable that malaria transmission is a locally closed circuit. No cattle are kept at this site, so that the factor of animal deviation can be ruled out.

The collector, sleeping within a trap-net, made collections therefrom at 2 a.m. and 6 a.m. After daylight all the 12 rooms were searched for resting adults as far as feasible. The three daily collections were sent each day in separate Barraud boxes for dissection at Calcutta.

The results to date are given in Table I.

The collector was withdrawn at the end of December, as the breeding season was obviously closing.

Of 206 *A. fluviatilis* caught in the trap-net from September to November, 33 per cent were taken at 2 a.m., when they were undoubtedly attempting to feed. These showed an oöcyst rate of 4.4 per cent, against a 2.9 per cent rate at 6 a.m. The solitary specimen with infective glands was taken at 6 a.m. Of the very small number of *A. varuna* taken in the trap-net in September and October, 20 per cent only were taken at 2 a.m.



TABLE I.

	JULY.			AUGUST.			SEPTEMBER.			OCTOBER.			NOVEMBER.			DECEMBER.		
	Num- ber dis- sected.	Oöcyst. rate.	Sporo- zoite rate.	Num- ber dis- sected.	Oöcyst. rate.	Sporo- zoite rate.	Num- ber dis- sected.	Oöcyst. rate.	Sporo- zoite rate.	Num- ber dis- sected.	Oöcyst. rate.	Sporo- zoite rate.	Num- ber dis- sected.	Oöcyst. rate.	Sporo- zoite rate.	Num- ber dis- sected.	Oöcyst. rate.	Sporo- zoite rate.
<b>I. In trap-net at 2 and 6 a.m.—</b>																		
<i>A. fluviatilis</i>	1	0.0	0.0	8	0.0	0.0	39	2.6	0.0	89	4.5	1.1	78	3.8	0.0	3	0.0	0.0
<i>A. varuna</i>	..	..	..	1	0.0	0.0	4	0.0	0.0	6	0.0	0.0	..	..	..	..	..	..
Percentage of <i>funestus</i> - group in catch.	7.7	..	..	9.8	..	..	29.7	..	..	85.6	..	..	89.7	..	..	50.0	..	..
<b>II. In rooms, early morning.—</b>																		
<i>A. fluviatilis</i>	3	0.0	0.0	9	0.0	0.0	69	7.3	1.5	613	3.9	2.3	583	1.5	2.1	24	0.0	0.0
<i>A. varuna</i>	1	0.0	0.0	..	..	..	15	0.0	0.0	33	3.0	0.0	..	..	..	..	..	..
Percentage of <i>funestus</i> - group in catch.	4.1	..	..	2.3	..	..	22.7	..	..	91.2	...	..	96.7	..	..	48	..	..



The proportion of *A. varuna* to the total *funestus*-group catch has been very low, 4 per cent as compared with 33 per cent in villages within 5 miles in the last 4 years (Senior White and Adhikari, 1940), but the sparsity of *A. varuna* is not reflected by the other member of the group, as in Chandsurat village, about 4½ miles away, the same collector, on the same date, in the same number of houses on each occasion took, on October 10, the height of the *funestus* season—

		1939.	1940.
<i>A. fluviatilis</i>	..	19	43
<i>A. varuna</i>	..	5	0

Evidently, local conditions in 1940 were unfavourable to the breeding of *A. varuna*. In regard to the daylight catch of specimens resting in rooms, the intention was to empty all the 12 rooms each day by catching. This was seldom possible, owing to individuals either going to work before dawn and locking their rooms, or leaving them unoccupied, but locked, for one or more days when they slept in the village. Observations at Darekasa, some 10 miles away, indicating that the *funestus*-group does not rest in totally unoccupied rooms, but in rooms occupied by night and locked before dawn introduce a disturbing factor into the following observations.

If the entire *funestus*-population rested by day in houses and there were no infiltration from the village, daily catching should eliminate all sporozoite carriers, and no more than young oöcysts should be found on dissection as soon as digestion is complete (48 hours up to mid-November, 72 hours in the colder weather). This has not been the case, sporozoites being to oöcysts as 27 : 38 (71 per cent), and sporozoite infections have followed oöcyst infections in crops at an average of 4·2 days, neglecting the size of the oöcysts found. (No data on the age of an oöcyst, with reference to size and to temperature, can be found in the literature.) Unless, therefore, all the sporozoite vectors have been resting in unsearched locked rooms, averaging 2·5 daily, then much resting of *A. fluviatilis* at least must occur out of doors, and this species, in the Satpuras, at any rate, is not fully susceptible to control by spray-killing of adults. *A. varuna* has been too rare this season for any deductions to be made as to its adult habits.

The experience gained in 1940 shows that for future investigations special arrangements should be made to ensure that every room can be searched daily. If outside resting is thus proved, the next step will be to clear all grass and undergrowth from around the area over an increasing distance, and study the effect of this on the resting habits and numbers collected of the two species.

### VIZAGAPATAM.

Around Vizagapatam, the problem is of a different nature. There is intense breeding of *A. varuna* in an area adjacent to the railway malaria control at Waltair station, but in spite of this the consolidated spleen rate of various hamlets forming Dondaparti village is not more than 3·9 per cent. Northward from Vizagapatam along the coast is a well-known healthy area where observations have been made in two large villages, Porlupalem and Venkatapalem, situated between Pendurti and Simhachalam railway stations. In both of these, the spleen rate was nil, though *A. varuna* was present in large numbers as shown in Table II.

Adult catches in human habitations showed that at Dondaparti the *funestus*-group is hardly ever present, whilst at Porlupalem, of all species resting by day,



from June to January, *A. fluviatilis* formed not more than 0·1 per cent and *A. varuna* 1·1 per cent, an entirely different state of affairs to that shown in Table I for the Satpuras. When, however, cattle-sheds were examined, the results were as given in Table III.

TABLE II.  
Percentage of all anopheline breeding.

Locality.	DONDAPARTI—WHOLE YEAR.		PORLUPALEM—JUNE TO JANUARY.	
	<i>fluviatilis</i> and <i>minimus</i> .	<i>varuna</i> .	<i>fluviatilis</i> and <i>minimus</i> .	<i>varuna</i> .
In kacha wells .. ..	0·7	91·7	0·9	70·0
In nalas and irrigation channels	1·8	71·2	0·3	9·8

TABLE III.  
Percentage of total anophelines collected in cattle-sheds.

Species.	February to August Dondaparti area.	Whole year Porlupalem area.
<i>A. fluviatilis</i> ..	1·6	..
<i>A. minimus</i> ..	1·9	0·1
<i>A. varuna</i> ..	65·4	45

The attraction of cattle-sheds for *A. varuna* is largely confined to a special type, a cone-shaped structure of palmyra-thatch carried down, save for the entrance, close to the ground. This type is common, but not universal, in this district, accounting for the difference in the percentage of *A. varuna* collected in the two groups of villages. In one such shed at Dondaparti, which was converted into a trap by carrying the thatching down to the ground and closing the door with a net at dawn, the catch has been as given in Table IV.

All the *A. varuna* and the two *A. minimus* dissections have proved negative. The malaria season in the locality is said by the villagers to be from January to March, but whatever be the vector it is not likely to be *A. varuna*, which around Vizagapatam appears to be purely a cattle feeder. A human-bait trap-net situated on the verandah of a pukka house within a few hundred yards of the cattle-shed caught no more than five *A. subpictus* and two *A. vagus* in 3 months.

In mid-December, a palmyra-thatch hut, of fresh material, was erected contiguous to the cattle hut, and baited by a man. The catches for 1½ months to the end of January are given in Table V.



TABLE IV.

*Female anophelines caught in one palmyra-thatch cattle-shed, Dondaparti.*

Month.	Number of catches.	<i>A. hyrcanus.</i>	<i>A. subpictus.</i>	<i>A. vagus.</i>	<i>A. culicifacies.</i>	<i>A. fluviatilis.</i>	<i>A. minimus.</i>	<i>A. varuna.</i>	<i>A. aconitus.</i>	<i>A. maculatus.</i>	<i>A. tessellatus.</i>	<i>A. ramsayi.</i>	<i>A. annularis.</i>	<i>A. pallidus.</i>	Total.	Percentage of <i>A. varuna.</i>
September	27	..	63	25	18	..	2	197	..	..	3	..	..	..	308	64.0
October ..	31	1	126	104	26	..	..	749	..	..	7	..	..	1	1,014	73.9
November	30	3	114	88	189	..	..	1,704	1	1	6	1	4	2	2,113	80.6
December	31	..	148	111	80	..	..	1,282	8	2	3	..	1	1	1,636	79.0
January ..	31	..	116	147	69	..	..	671	3	..	1	..	..	..	1,007	66.6
TOTAL ..	..	4	567	475	382	..	2	4,603	12	3	20	1	5	4	6,078	75.4

TABLE V.

*Female anophelines caught in the palmyra-thatch human-bait hut, Dondaparti.*

Month.	Number of catches.	<i>A. hyrcanus.</i>	<i>A. subpictus.</i>	<i>A. vagus.</i>	<i>A. culicifacies.</i>	<i>A. fluviatilis.</i>	<i>A. minimus.</i>	<i>A. varuna.</i>	<i>A. aconitus.</i>	<i>A. maculatus.</i>	<i>A. tessellatus.</i>	<i>A. ramsayi.</i>	<i>A. annularis.</i>	<i>A. pallidus.</i>	Total.	Percentage of <i>A. varuna.</i>
December	16	..	79	82	24	..	..	73	1	1	..	..	..	..	260	28.1
January	31	..	200	318	38	..	..	81	1	..	..	..	..	..	638	12.7

It was noticed that the specimens of *A. varuna* were resting chiefly on the side of the hut contiguous to the cattle hut, and lest they should actually be passing through the palmyra-thatch (into which they penetrate deeply by day), special precautions were taken from January. The much smaller percentage of *A. varuna* in the catches with human bait is very striking. All dissections of *A. varuna* were negative, as were also 81 dissections of *A. vagus* made during a few days when this species was particularly prevalent. It appears that, though, at Dondaparti, *A. varuna* is very difficult to obtain in ordinary houses, it can be taken in some numbers in palmyra-thatch huts, but such are not normally human habitations. In these, thatching is ordinarily used for the roof, but the walls are of mud.



As the work around Vizagapatam is being carried out by the railway staff, this part of the investigation will not be interrupted. It may be that *A. fluviatilis* and/or *A. minimus* only are the local vectors, or it may be quite another species such as *A. stephensi*, which we have recently found with sporozoites in 3 out of 11 specimens in an epidemic at Saripalli village near Pendurti station, normally a typical village of the healthy coastal plain\*. The race concerned (but eggs were obtained from negative specimens only) was var. *mysorensis*.

Now *A. varuna* is a proved vector in the hills of East Central India, and in Deltaic Bengal (Iyengar, 1928; Roy, 1939). In the hope that the maxillary index of *A. varuna* from these localities and from around Vizagapatam would show a difference (the figure of 12.1 given in Senior White, 1937, is a composite of specimens collected in all three areas), additional material is being collected to complete 200 maxillæ from each area. The results so far obtained are given in Table VI.

TABLE VI.  
Maxillary counts—*A. varuna*.

Locality.	10.	11.	12.	13.	14.	15.	16.	Total.	Maxillary index.
Vizagapatam ..	2	21	54	73	36	13	1	200	12.8
Hooghly Delta ..	7	29	84	57	15	8	..	200	12.3

The difference is not statistically significant, nor is the dispersion ( $P = 0.2$ ). Sufficient material from the hills has not yet been obtained, but it would appear that though the species is a vector near Calcutta and not near Vizagapatam, the difference in constitution is not one involving the maxillary index.

In identifying *A. varuna* material from the Satpuras and from Vizagapatam prior to dissection, it was noticed that in the former the thoracic dorsum is greyish and in the latter more brownish, whilst the first black palpal band appears definitely broader in the hills than on the coast. This latter point is susceptible of statistical examination, and unrubbed bred out material from both areas is now being accumulated.

#### REFERENCES.

- IYENGAR, M. O. T. (1928) .. Report on the malaria survey of the environs of Calcutta. Bengal Sec. Book Depôt, Calcutta.  
 ROY, D. N. (1939) .. The importance of *A. varuna* Iyengar as a carrier of malaria in Bally (Calcutta). *J. Mal. Inst. Ind.*, **2**, 3, pp. 239-242.  
 SENIOR WHITE, R. A. (1937) .. The maxillary index of the Indian anophelines. *Rec. Mal. Surv. Ind.*, **7**, 2-3, pp. 155-177.  
 SENIOR WHITE, R. A., and ADHIKARI, A. K. (1940). On malaria transmission in the Eastern Satpura Ranges. *J. Mal. Inst. Ind.*, **3**, 4, pp. 383-412.

\* The epidemic occurred in November. The child spleen rate, taken in February, was 74 per cent.



# STUDIES ON THE BEHAVIOUR OF *ANOPHELES MINIMUS*.

## Part IV.

### THE COMPOSITION OF THE WATER AND THE INFLUENCE OF ORGANIC POLLUTION AND SILT.

BY

R. C. MUIRHEAD THOMSON.

(*Research Worker supported by the Royal Society and the London School of Hygiene and Tropical Medicine.*)

[March 1, 1941.]

## CONTENTS.

	PAGE.
I. THE COMPOSITION OF THE WATER AND THE INFLUENCE OF ORGANIC POLLUTION .. .. .	64
A. INTRODUCTION .. .. .	64
B. METHODS AND GENERAL CONSIDERATIONS .. .. .	64
C. THE COMPOSITION OF THE WATER .. .. .	66
(1) Dry weather breeding places .. .. .	66
Oxygen .. .. .	66
Organic matter .. .. .	68
(2) Monsoon breeding places .. .. .	71
Organic matter .. .. .	75
(3) Reactions of <i>A. minimus</i> to different natural waters .. .. .	76
Gravid females .. .. .	76
Larvæ .. .. .	77
D. EXPERIMENTAL POLLUTION OF ANOPHELINE BREEDING PLACES WITH VEGETATION .. .. .	78
(1) Course of pollution in laboratory .. .. .	78
(2) Course of pollution in field .. .. .	79
(3) The effect of vegetable pollution on the breeding places of <i>A. hyrcanus</i> .. .. .	82
E. THE REACTIONS OF <i>A. minimus</i> TO POLLUTED WATER .. .. .	85
(1) Gravid females .. .. .	85
(2) Larvæ .. .. .	89
F. THE POLLUTION OF <i>A. minimus</i> BREEDING PLACES WITH ROTTING VEGETATION .. .. .	90
(1) Herbage packing .. .. .	90
(2) Pollution of tea garden drains with factory effluent .. .. .	90
Field experiments .. .. .	90
Reactions of gravid females .. .. .	94
Reactions of larvæ .. .. .	96



II. SILT	..	..	..	..	..	..	96
INTRODUCTION	..	..	..	..	..	..	96
MEASUREMENT OF TURBIDITY	..	..	..	..	..	..	97
FIELD EXPERIMENTS	..	..	..	..	..	..	97
LABORATORY EXPERIMENTS	..	..	..	..	..	..	98
DISCUSSION	..	..	..	..	..	..	99
SUMMARY	..	..	..	..	..	..	100
REFERENCES	..	..	..	..	..	..	101

## I. THE COMPOSITION OF THE WATER AND THE INFLUENCE OF ORGANIC POLLUTION.

### A. INTRODUCTION.

THE composition of the water has long been recognized as a factor of great importance in the ecology of anopheline mosquitoes, and a considerable amount of work has been done, notably by Senior White (1926; 1928) and Beattie (1930; 1932), in an endeavour to explain, in terms of water chemistry, the limitations of many species to certain kinds of water.

For this aspect of ecology, *A. minimus* seems a particularly suitable subject for experiment, as its breeding places are for the most part confined to the grassy edges of streams, open drains and rivers, and its larvæ are very seldom found in the still waters of tanks, borrowpits, swamps and stagnant ricefields. The existence of such a clearly defined type of breeding place makes it seem possible that there is some difference in the character of the water itself involved. In the three previous papers of this series (Thomson, 1940a; 1940b; 1940c), the parts played by light and shade, water movement, and temperature on the selection of the breeding place were studied by experimental methods. In the present investigation, the same general methods have been applied to study the composition of the water and the influence of organic pollution on the behaviour of this species.

All the chemical analyses were carried out by the author at the Tocklai Experimental Station of the Indian Tea Association in Upper Assam, and he is greatly indebted to the Director and to various members of the Chemical Branch for much useful help and guidance.

### B. METHODS AND GENERAL CONSIDERATIONS.

The physical factors which have been dealt with in the previous papers of this series have all been fairly simple and definite, capable of being measured in the field and of being controlled and varied independently of each other in the laboratory. In extending experimental methods to the composition of the water, we are no longer dealing with a single factor but with a mass of variable characters, some of which can be measured accurately, but others which can only be estimated approximately by artificial standards. To make a complete analysis of every sample of water, it would be necessary to devote our whole time to chemistry alone, and no attempt, therefore, has been made to do so in the present investigation. The fact that the breeding of *A. minimus* is mostly confined to the edges of clear, unpolluted, running water suggests that attention should be directed first to the purity of the water and the amount of organic matter it contains. There is no simple, straightforward method of estimating 'dissolved organic matter' or the amount of organic pollution in water, but by means of a few standard methods it is possible to form a good idea of the



comparative degree of pollution and the standard of purity of the water. The following analyses were carried out :—

<i>Free and saline ammonia</i>	}	These were both carried out by the standard distillation method.
<i>Albuminoid ammonia</i>		

*Oxygen absorbed from acid permanganate (Tidy figure).*—This analysis is widely used in public health to give an indication of the amount of oxidizable organic matter in the water. The usual convention is to incubate the sample for 4 hours at 27°C., but different conventions are used by different chemists; as the temperature of standing water in the laboratory in Upper Assam is well over 27°C. for many months of the year, the convention used throughout this work is incubation for 4 hours at 40°C. The Tidy figure has been little used in previous work on anopheline ecology and yet it has proved, as later figures will show, to be one of the most useful figures for indicating in itself the nature of the water in the breeding place. The method of analysis is simple and it is possible to test many samples of water at the same time, depending on the size of the constant-temperature oven or water-bath.

*Dissolved oxygen.*—A short series of determinations of dissolved oxygen was carried out by the Winkler method. This method is not reliable in water containing various oxidizing and reducing agents, notably nitrites and ferrous salts. According to Skopintzev and Ovehinnikova (1933), however, the method is valid provided the nitrite content is not more than 0.1 mg. per litre, and the amount of ferrous iron not more than 25 mg. per litre. Of the various samples of water collected from anopheline breeding places, not a single one was found to contain nitrites or ferrous iron, an experience similar to that of Iyengar (1930). The only time when nitrite was detectable was in a drain polluted by factory effluent. The series of dissolved oxygen determinations in this paper is, therefore, unaffected by either nitrite or ferrous iron. Probably owing to the presence of one or both of these substances, Senior White (1928) had to consider his dissolved oxygen figures as quite unreliable; the difficulty, however, is not insuperable and can be overcome, even in the presence of large amounts of nitrite, by introducing slight modifications of the Winkler method as shown by Skopintzev and Ovehinnikova (*loc. cit.*), Brandt (1937), Alsterberg (1925) and Stas (1925).

*Nitrites.*—These were measured by the Greiss-Ilosvay method. Nitrites were absent from all anopheline breeding places tested.

*Nitrates.*—A few determinations of nitrates were made by phenoldisulphonic acid, but in most cases the amount of organic matter in the water was great enough to upset the colour comparisons.

*Ferrous iron.*—This was tested with potassium ferrieyanide. It was absent from all samples tested.

Concentrating, therefore, on those analyses which give an estimate of the dissolved organic matter and the degree of pollution of the water, the enquiry was made along the following lines. First, is there a constant difference in composition between the water from the normal breeding place of *A. minimus* and that of stagnant water where this species is seldom found? In the dry months of the year this problem resolves itself into a comparison between the water in perennial rivers and the water in fresh-water tanks, while during the monsoon season the comparison will be between open garden drains on the



one hand and various types of still water, particularly ricefields, on the other. If there is a significant difference in composition, it will be necessary to find out if it is appreciated by the female mosquito when selecting a breeding place, and if it has any effect on the growth of larvæ. Secondly, the problem will be tackled from another direction by making an experimental study of control of breeding places by pollution and herbicide packing, to find out how these methods work and what degree of pollution is necessary to prevent breeding. In much previous work, it has been tacitly assumed that control in such cases is brought about by a lethal or inhibitory effect on the larvæ; but the problem, as has been repeatedly stressed in this investigation, is of a two-fold nature. Any artificial alteration in the composition of the breeding place will have to be considered, not only from the point of view of the larva, but also in the way it influences the behaviour of the female mosquito when selecting a breeding place.

### C. THE COMPOSITION OF THE WATER.

#### (1) *Dry weather breeding places.*

During the dry weather from December till April, the grassy edges of perennial rivers become the main breeding places of *A. minimus* (Plate XV, fig. 1). During most of this period, the only other anopheline breeding places of note are numerous fresh-water tanks (Plate XV, fig. 2; Plate XVI, figs. 3 and 4) which contain water throughout the year, but are seldom used by *A. minimus*.

*Oxygen.*—It seemed natural to suppose that the river water would always be better aerated than the stagnant water in the tanks, and that such a difference might be used by the female mosquito to discriminate between different waters. Iyengar (*loc. cit.*), in his detailed study of the dissolved oxygen of stagnant ponds and ditches in relation to anopheline breeding, found no evidence of any 'oxygen toleration limits' for six species of *Anopheles*. He also found that the surface water of ponds showed remarkable variations in the concentration of dissolved oxygen at different periods of the day, and the existence of a similar diurnal variation of oxygen in river water has been demonstrated by Butcher *et al.* (1927*a*; 1927*b*), the percentage saturation varying from 65 to 157.

A series of dissolved oxygen determinations was carried out, during March and April, in a river and two selected tanks; Tank 1 had a lot of vegetation, floating and submerged, particularly round the edges, while Tank 2 had bare edges with most of the vegetation submerged. The samples of water were collected from just below the surface, the usual precautions being taken to ensure that the sample had no contact with atmospheric air during collecting. The apparatus used was very similar to that of Iyengar (*loc. cit.*) and the manganous chloride and alkaline potassium iodide were added in the field immediately after collection.

Preliminary tests having shown that the oxygen content fluctuated considerably during the day, samples were collected and compared at three different periods, roughly at 10 a.m. in the morning, 2 p.m. in the afternoon, and 8 to 10 p.m. at night. The figures are shown in Table I.

The results, which are quite contrary to what was expected, show that there is no constant difference in oxygen content between running water and stagnant water; the percentage saturation in the perennial river varied from 88 to 107, while that of the tanks varied from 66 to 140, both having their maxima



PLATE XV.



Fig 1 Perennial river, cold weather breeding place of *A. minimus*



Fig 2. Tank 10.



PLATE XVI.



Fig 3 Tank 11

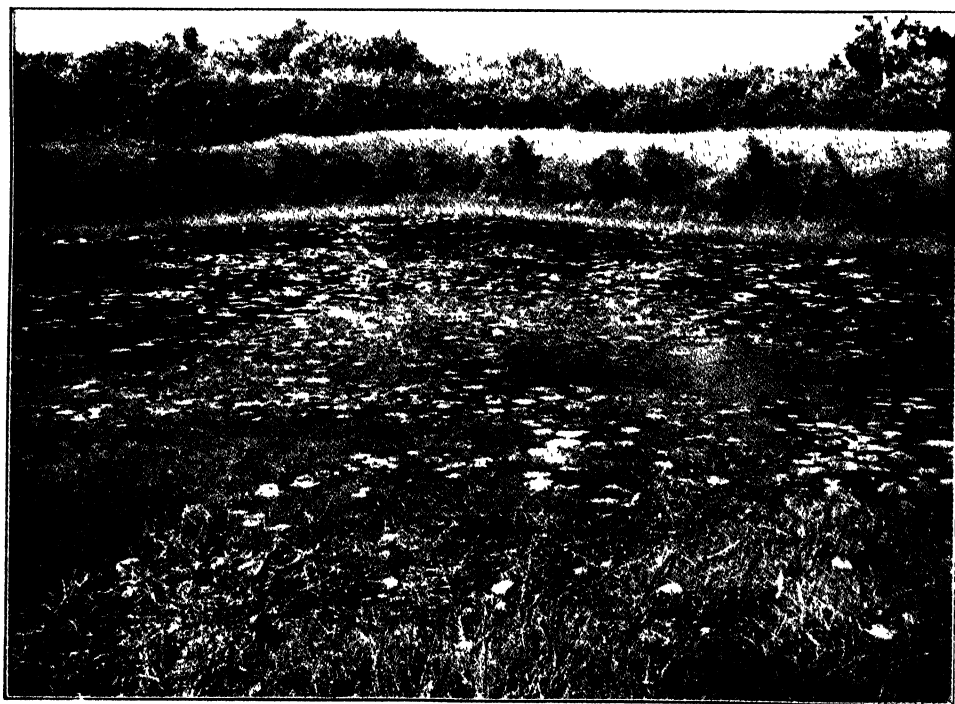


Fig. 4. Tank 12



TABLE I.

*Concentration of dissolved oxygen in river water and tank water.*

Date.	Locality.	Time.	Water temperature.	Oxygen mg. per litre.	Oxygen percentage saturation.
18/iii	River	10-45 a.m.	23.5°C.	7.56	89
	Tank 1	10-30 a.m.	25.0°C.	7.32	89
	River	2-15 p.m.	28.0°C.	7.72	99
	Tank 1	2-30 p.m.	31.0°C.	10.35	140
	Tank 2	2-30 p.m.	29.5°C.	8.53	112
	River	8-00 p.m.	26.5°C.	7.19	90
	Tank 1	8-15 p.m.	27.0°C.	6.61	83
	Tank 2	8-15 p.m.	26.5°C.	8.39	104
19/iii	River	10-00 a.m.	24.0°C.	8.34	99
	Tank 1	10-15 a.m.	26.2°C.	8.36	104
	Tank 2	10-15 a.m.	25.5°C.	8.31	102
	River	2-00 p.m.	27.2°C.	7.94	100
	Tank 1	2-15 p.m.	27.5°C.	10.26	130
	Tank 2	2-15 p.m.	26.5°C.	9.23	115
	River	8-15 p.m.	24.2°C.	7.64	91
	Tank 1	8-30 p.m.	24.0°C.	6.59	78
	Tank 2	8-30 p.m.	24.5°C.	7.90	95
21/iii	River	10-15 a.m.	22.5°C.	8.83	102
	Tank 1	10-30 a.m.	24.2°C.	8.88	106
	Tank 2	10-30 a.m.	24.8°C.	8.03	97
	River	2-15 p.m.	27.5°C.	8.44	107
	Tank 1	2-30 p.m.	28.0°C.	9.65	124
	Tank 2	2-30 p.m.	27.2°C.	8.82	111
	River	8-30 p.m.	24.0°C.	7.62	91
	Tank 1	8-45 p.m.	24.5°C.	7.76	93
	Tank 2	8-45 p.m.	24.2°C.	9.27	110



TABLE I—*concl'd.*

Date.	Locality.	Time.	Water temperature.	Oxygen mg. per litre.	Oxygen percentage saturation.
* 31/iii	River	10-00 p.m.	27.5°C.	6.95	88
	Tank 1	9-30 p.m.	27.5°C.	8.28	105
	Tank 2	9-30 p.m.	27.5°C.	8.60	109
† 1/iv	River	9-00 a.m.	25.0°C.	7.61	92
	Tank 1	9-30 a.m.	26.0°C.	5.46	66
	Tank 2	9-30 a.m.	25.0°C.	5.93	72

\* A warm night after a very hot bright day.

† A dull cloudy day.

2 to 3 hours after noon. The range of variation is greater in the tanks than in the river, and they frequently become super-saturated during the day. The samples of 31/iii show that, after a very hot day, the tank water may still be super-saturated at 10 p.m. at night. It seems probable that the oxygen content of the tanks may fall below that of the river later in the night, but this point was not investigated. As the figures were sufficient to show that no constant difference existed either by day or in the early part of the night, and that the oxygen content in still water over vegetation is subject to great fluctuations according to the amount of sunlight, it was not considered worth while to make a more detailed investigation. In view of such variations, it is evident that the dissolved oxygen content can be of little use in helping the female mosquito to distinguish different kinds of natural water.

*Organic matter.*—Since there was no constant difference between the oxygen content of running and stagnant water, attention was turned to the dissolved organic matter and degree of pollution. In the early, cool part of the dry season, in November and December, a series of routine analyses was carried out in a perennial river (Plate XV, fig. 1) and four representative fresh-water tanks 10, 11, 12 and 16 (Plate XV, fig. 2; Plate XVI, figs. 3 and 4). During this period there were no heavy falls of rain and the river ran clear all the time. The analyses made were of free and saline ammonia, albuminoid ammonia, and the Tidy figure (oxygen absorbed from permanganate at 40°C.). The temperature of the river water at the time of sampling varied from 21.5°C. to 17.0°C. In the ammonia distillation, 500 c.c. of river water and 250 c.c. of tank water were sampled each time. The results are set out in Tables II (i) and III. It is seen that free and saline ammonia were absent from all samples collected both in the river and in the tanks, and this test is obviously useless for distinguishing different kinds of breeding place. On the other hand, the results of the albuminoid ammonia test and of the Tidy figure are very striking. The composition of the river water remains fairly constant for both these factors, and the figures remain lower than those of tank waters. The differences were as follows:—The mean Tidy figure of 20 samples of perennial river was 0.85 parts per million, while the mean Tidy figure of 35 samples of tank water was







TABLE III.

*Free and saline and albuminoid ammonia in tanks and perennial river, in parts per million. November to December.*

Date.	PERENNIAL RIVER.		TANK 10.		TANK 11.		TANK 12.		TANK 16.	
	Free amm.	Alb. amm.	Free amm.	Alb. amm.	Free amm.	Alb. amm.	Free amm.	Alb. amm.	Free amm.	Alb. amm.
22/xi	0	0.097	..	..	..	..	..	..	..	..
26/xi	0	0.030	0	0.270	..	..	..	..	..	..
27/xi	0	0.050	..	..	0	0.250	..	..	..	..
28/xi	0	0.040	0	0.320	..	..	..	..	..	..
29/xi	0	0.050	..	..	0	0.170	..	..	..	..
30/xi	0	0.050	..	..	..	..	..	..	0	0.230
2/xii	0	0.050	0	0.320	..	..	..	..	..	..
3/xii	0	0.050	..	..	..	..	0	0.250	..	..
4/xii	0	0.040	..	..	..	..	..	..	0	0.180
5/xii	0	0.040	..	..	..	..	0	0.160	..	..
6/xii	0	0.040	..	..	..	..	..	..	0	0.220
9/xii	0	0.030	..	..	..	..	..	..	0	0.160
10/xii	0	0.036	..	..	..	..	0	0.178	..	..
11/xii	0	0.066	..	..	..	..	..	..	0	0.266
12/xii	0	0.034	..	..	..	..	0	0.180	..	..
13/xii	0	0.044	..	..	..	..	..	..	0	0.250
14/xii	0	0.040	..	..	..	..	..	..	0	0.210
16/xii	0	0.036	..	..	..	..	0	0.228	..	..
17/xii	0	0.033	0	0.242	..	..	..	..	..	..
18/xii	0	0.034	..	..	0	0.180	..	..	..	..
19/xii	0	0.033	..	..	0	0.160	..	..	..	..
20/xii	0	0.047	0	0.180	..	..	..	..	..	..
21/xii	0	0.020	..	..	0	0.160	..	..	..	..
Mean	..	0.043	Mean of all tanks.		0.217					



2.58 parts per million. The mean albuminoid ammonia figure of 23 samples of river water was 0.043 parts per million, while the mean albuminoid ammonia figure of 22 samples of tank water was 0.217 parts per million. The mean Tidy figure for the tanks is about three times, and the albuminoid ammonia five times greater than the corresponding figures for the river. By applying a convenient formula used in public health, we can combine these two analyses and obtain an index of the 'degree of pollution' as follows:—

$$\frac{(\text{Albuminoid ammonia} \times 100) + (\text{Tidy figure} \times 10)^*}{2}$$

This gives us a figure of 0.64 for perennial river and 2.38 for tanks, and shows that during this period the mean degree of pollution of tanks is 3.7 times greater than that of the perennial river.

A further series of analyses was made at the end of the dry season in April and May. During April there was no heavy rain, and the level of river and tanks was lower than before. The temperature of the river water varied from 24.5°C. to 28.5°C. at the time of sampling. With the first heavy fall of rain in May, the river came down in spate, and from that time onwards this occurred at frequent intervals after heavy showers. The results of these analyses are shown in Tables II (*n*) and IV. From these data, it will be seen that the Tidy figures during April are higher in both river and tanks than they were in the early part of the dry season, but that the mean figure for tanks, 5.51, is still higher than that of river water, 2.03. There are no albuminoid ammonia figures available for this month, but it is very likely that they follow the same general trend. With the onset of heavy rain in May and June, the albuminoid ammonia and the Tidy figure of tanks are not greatly affected, but in the river water considerable fluctuations are apparent, and there is no longer a constant difference in composition between river and tank water. Each time the river comes down in flood, there is a great increase in the degree of pollution in the water, as reflected in the Tidy figures of 9/v and 21/v and the albuminoid ammonia figures of 20/v and 10/vi. As the level of the river slowly falls in the intervals between heavy showers, there is a corresponding fall in the Tidy and albuminoid ammonia figures, until the next heavy shower brings about a sharp increase in both figures, and as much as a five-fold increase in albuminoid ammonia may occur as on 4/vi and 10/vi. It is interesting to note that these drastic changes have no effect on the free and saline ammonia figure, which is either completely absent or only present as traces.

## (2) Monsoon breeding places.

During and after the monsoon, from June till November, the typical breeding place of *A. minimus* is along the grassy edges of small streams and open drains. In this district, the tea garden drains were particularly favourable and continuous dense breeding was liable to occur there. During this period, the most extensive areas of still water were the innumerable stagnant, rain-filled ricefields, in which *A. minimus* larvæ were practically never found to occur. A series of routine analyses was carried out from June till November to find out if there was any difference in the composition of water from ricefields

---

\* The formula refers to parts per 100,000 while all figures in this paper are in parts per million.



and that of garden drains, in the same way as the perennial river was compared with tanks in the dry weather. The samples were taken from six garden drains, while the ricefields were selected at random each time. While the garden drains

TABLE IV.

*Free and saline and albuminoid ammonia in perennial river and tanks, in parts per million. May and June.*

Date.	PERENNIAL RIVER.		TANK 10.		TANK 11.		TANK 16.	
	Free amm.	Alb. amm.	Free amm.	Alb. amm.	Free amm.	Alb. amm.	Free amm.	Alb. amm.
8/v	..	..	..	..	..	..	0.000	0.273
9/v	..	..	..	..	0	0.393	..	..
10/v	..	..	0.000	0.589	..	..	..	..
13/v	0	0.280	..	..	..	..	..	..
14/v	0	0.172	..	..	..	..	..	..
15/v	0	0.124	..	..	..	..	..	..
16/v	..	..	..	..	0	0.327	..	..
17/v	..	..	..	..	..	..	0.010	0.368
18/v	..	..	0.026	0.424	..	..	..	..
20/v	0	0.477	..	..	..	..	..	..
21/v	..	..	..	..	..	..	..	..
22/v	..	..	..	..	0	0.412	..	0.339
23/v	..	..	0.000	0.582	..	..	0.000	..
24/v	0	0.444	..	..	..	..	..	..
27/v	0	0.214	..	..	..	..	..	..
30/v	0	0.201	..	..	..	..	..	..
4/vi	0	0.126	..	..	..	..	..	..
5/vi	..	..	..	..	..	..	0.000	0.400
7/vi	..	..	0.000	0.888	..	..	..	..
10/vi	0	0.666	..	..	..	..	..	..

remained very much the same in appearance during this period, the ricefields underwent great changes according to the stage of cultivation, and this is reflected in the fluctuations in the composition of the water. The first series of samples were taken from fallow fields before ploughing; later the samples came







TABLE VI.

*Free and saline and albuminoid ammonia in ricefields and garden drains, in parts per million. June to November.*

Date.	GARDEN DRAINS		RICEFIELDS.		Date.	GARDEN DRAINS.		RICEFIELDS.	
	Free amm.	Alb. amm.	Free amm.	Alb. amm.		Free amm.	Alb. amm.	Free amm.	Alb. amm.
3/vi	.	..	0	0.678	11/ix	0.000	0.080	..	..
6/vi	..	..	0	0.873		0.240	0.180	..	..
14/vi	..	..	0	0.800	12/ix	..	..	0.000	0.380
			0	0.525				0.000	0.520
2/vii	0.000	0.180	..	..	14/ix	0.128	0.112	..	..
10/vii	0.000	0.256	..	..		0.000	0.137	..	..
30/vii	0.000	0.190	..	..	17/ix	0.210	0.112	..	..
	0.000	0.296	..	..		0.000	0.096	..	..
2/viii	0.000	0.228	..	..	19/ix	0.000	0.128	..	..
9/viii	..	..	0	0.600	21/ix	..	..	0.136	0.606
13/viii	0.070	0.249	..	..				0.076	0.540
	0.000	0.205	..	..	27/ix	0.200	0.160	..	..
14/viii	..	..	0	0.409		0.000	0.170	..	..
17/viii	0.000	0.195	..	..	3/x	..	..	0.000	0.276
	0.000	0.137	..	..				0.000	0.420
20/viii	0.000	0.144	..	..	8/x	0.000	0.080	..	..
27/viii	0.000	0.144	..	..	18/x	0.040	0.030	..	..
30/viii	0.000	0.134	..	..	4/xi	0.070	0.128	..	..
31/viii	..	..	0	0.432		0.000	0.100	..	..
			0	0.897	Mean		0.155		0.590
3/ix	0.000	0.237	..	..					
5/ix	0.000	0.185	..	..					
9/ix	..	..	0	0.868					
			0	0.614					



from fallow fields, freshly ploughed fields, and those with recently transplanted seedlings, while the final samples were taken from fields in which the rice plants had grown sufficiently to keep the water shaded and cool.

*Organic matter.*—Since the albuminoid ammonia and Tidy figures had proved so useful in comparing river water with tank water, particular attention was again paid to these two analyses for the comparison of ricefields and garden drains. The results are shown in Tables V and VI. The mean Tidy figure of 33 samples of drain water was 2.57 parts per million. The mean Tidy figure of 47 samples of ricefield water was 6.67 parts per million. The mean albuminoid ammonia figure of 27 samples of drain water was 0.155 parts per million, while the mean albuminoid ammonia figure of 16 samples of ricefield water was 0.590 parts per million.

Combining these by means of the formula as before

$$\frac{(\text{albuminoid ammonia} \times 100) + (\text{Tidy figure} \times 10)}{2}$$

we find that the mean 'degree of pollution' of garden drains is 2.06, compared with a figure of 6.28 for ricefields. During this period, therefore, the mean degree of pollution for ricefields is three times greater than that of garden drains. It will also be noticed that the figures in general, during this hot wet period, are much higher than in the cool dry season, but that a striking difference in composition between stagnant and clear running water is still maintained.

While it is better to combine the albuminoid ammonia figure and the Tidy figure in one formula giving an estimate of the degree of pollution or amount of organic matter, it is seen that each factor in itself gives an almost equally good indication of the difference in composition between the two types of water. It is not always feasible to do a large number of ammonia distillations, and in such cases the Tidy figure by itself is a valuable index of dissolved organic matter. During the greater part of the monsoon, this figure is constantly higher in ricefields than in garden drains. Towards the end of the season, however, with the growth of the rice plants and consequent shading and cooling of the water, the figure may occasionally approximate to that of garden drains as in the analyses of 27/ix and 11/xi (Table V). It is probable that this may also happen with albuminoid ammonia, although the figures are not complete enough to show whether this is the case or not.

The complete absence of free and saline ammonia from most of the ricefields and garden drains, together with the high figure for albuminoid ammonia, supports and amplifies the results obtained in the dry weather. All the records of free and saline ammonia occurring in a garden drain refer to the same drain, which flowed through coolie lines and was obviously exposed to pollution by animal or human excrement. The fact that in such cases the free ammonia figure was usually higher than the albuminoid ammonia one, is an indication of recent pollution by animal organic matter. Where no such source of animal pollution existed, free ammonia was never present in more than a trace. A similar almost complete absence of free ammonia in the presence of considerable quantities of albuminoid ammonia and oxidizable organic matter has been recorded in a South Indian lake by Ganapati (1940).

All these figures for ammonia are in striking contrast to those obtained by Senior White (1928), in the Delhi urban area. From his constantly high figures for free ammonia, frequently exceeding those of albuminoid ammonia, it appears



that most of the breeding places were polluted with animal organic matter (? sewage). Senior White has suggested that 'saline ammonia is inhibitory to anopheline breeding, save in the case of the *rossi* group, in amounts exceeding one part per million'; and Beattie (1932) has shown a correlation between ammonia nitrogen and anopheline prevalence in Trinidad, on oviposition more than on the life of the larvæ. These results show the existence of conditions very different from those encountered in the present investigation; the figures obtained in Assam may be considered fairly representative of rural conditions in that part of India, and they show that the free and saline ammonia figure is quite valueless as an indicator of the difference between different kinds of anopheline breeding place.

De Jesus (1936), from a long series of analyses, showed that the breeding places of *A. minimus* var. *flavirostris* had low concentrations of ammonia nitrogen, albuminoid nitrogen, nitrite nitrogen, and nitrate nitrogen. He found that this species occurred mostly in concentrations of 0.050 to 0.349 parts per million of albuminoid nitrogen, which compares favourably with the range of 0.020 to 0.296 found in Assam.

A low concentration of albuminoid nitrogen has been associated with the breeding places of *A. maculatus* by Hacker (1923), and Williamson (1928; 1936) has stressed the probable importance of the forms of nitrogen and of the intermediate decomposition products of proteins in the ultimate determination of the breeding place. Recently, Kapeszky (1940) has found that the limiting ammonia nitrogen concentration beyond which *A. maculipennis* and *A. bifurcatus* do not occur is 0.4 and 0.25 parts per million respectively.

The existence of such a striking measurable difference between the composition of clear running water favoured by *A. minimus* and the water from stagnant tanks and ricefields seems to suggest that water composition is a factor of first importance in this case. In fact, there is a great temptation to say that this is the vital limiting factor in the determination of the breeding place. But before jumping to immediate conclusions, it will be necessary to apply experimental methods to clarify the problem. We will have to find out if the difference in composition can be appreciated by the female mosquito when it is selecting a breeding place, and if it has any effect on the growth or mortality of larvæ.

### (3) Reactions of *A. minimus* to different natural waters.

*Gravid females*.—A series of laboratory experiments was carried out in which freshly caught *A. minimus* females were exposed to a choice of waters in which to lay their eggs. The alternatives were river water compared with tank water, tank water with a low degree of pollution compared with tank water with a high degree of pollution, and water from garden drains compared with tank water. In this simple kind of experiment, it was found necessary to carry out several repeats, involving a large number of eggs, before any valid conclusions could be arrived at. The figures from single experiments might give the most misleading results; for example, the first experiment might show a strong preference for one kind of water, while on a second night there might be an equally strong preference for the other water. In all these experiments, the behaviour of the female mosquitoes was very erratic and there was no consistent preference to lay eggs in the water from the breeding place. For example, in four experiments in which they were given the choice of laying their



eggs in water from a garden drain and water from a tank and a borrowpit, the results were as follows:—

Drain water.	Tank and borrowpit water.
1,852	816
567	845
852	312
574	1,851
<hr/> 3,845 <hr/>	<hr/> 3,824 <hr/>

Out of 7,669 eggs, 3,845 or 50·1 per cent were laid in drain water. From these and similar experiments it appears that the ovipositing female is not able to distinguish between the water from garden drains and the water from stagnant tanks and borrowpits in which it never breeds. When selecting a breeding place, the behaviour is evidently not affected by the range and *type* of organic matter encountered in these different kinds of water. Exactly what amount and type of organic pollution is appreciated by the female will be discussed later in this paper. The composition of the water is not, therefore, the vital factor in the selection of the breeding place, and the low degree of pollution of clear running water, whatever other function it may serve, is not the character which ultimately determines the behaviour of the mosquito.

It remains to be seen whether the difference in composition of water has any effect on the growth and mortality of larvæ.

*Larvæ.*—A series of experiments was carried out to find if water from the natural breeding place was more suitable for growth of *A. minimus* larvæ than water from other anopheline breeding places. It was always found difficult in the laboratory to rear *A. minimus* from the egg to the adult stage, and in the following experiments it was seldom possible to carry the experiment through to its natural conclusion. Newly hatched larvæ, in batches of 100 or 200, were put in large dishes containing water from the perennial river and from different tanks. Larvæ were counted and the water changed every second or third day; all the handling of eggs and young larvæ was carried out with the thin film of water on a wire loop. In all water, there was a heavy mortality and each experiment was concluded after 3 or 4 weeks when the number of larvæ in all dishes became very low. From a long series of experiments, no positive conclusions could be drawn; the cultures of larvæ behaved erratically, and it was soon apparent that the growth and mortality in river water did not differ markedly from that in different tank waters. There was no relation between the behaviour of the larvæ and the composition and different degree of pollution of the water; in fact in one experiment, from 200 newly hatched larvæ kept in water from a weedy tank (10), 30 pupæ were obtained by the end of 3 weeks, showing that there is nothing particularly toxic about such water with a high albuminoid ammonia and Tidy figure. Further evidence that there is no relation between the organic matter or degree of pollution of the water and its suitability for *A. minimus* larvæ will be given later in this paper.

Since the low degree of pollution of clear running water has not proved itself to be a factor of any great significance in the determination of the



breeding place, attention will now be turned to the effects of artificial and experimental pollution.

#### D. EXPERIMENTAL POLLUTION OF ANOPHELINE BREEDING PLACES WITH VEGETATION.

The absence of many anophelines from heavily polluted water has long been appreciated, and the application of this fact in the deliberate pollution of breeding places is now a well established naturalistic method of control (Williamson, 1928; 1935; 1936) and Hackett *et al.* (1938). Unfortunately, there is very little information on the exact rôle of organic pollution and how such methods of control really act. Although the following investigation is mainly concerned with *A. minimus*, many of the principles involved may eventually be of wider application.

The first stage is to find out what happens when vegetation and cut jungle decompose in water, and if it is possible to follow the changes which take place by means of standard chemical methods.

##### (1) *Course of pollution in laboratory.*

For this purpose three of the most common wayside bushes were used as follows\*: *Melastoma melasthricum*, common by the road-side and on grazing ground, *Eugenia balsamia*, common by road-side and on waste ground, and *Eupatorium odoratum*, the most common plant at the edge of jungles, along the wayside, and in all places where jungle has recently been cleared. It is particularly common round tea gardens and is one of the plants used in control of *A. minimus* by 'shade'.

Branches and leaves of these plants were cut off and immersed in large dishes full of water. No definite amounts or proportions were used, but each dish contained roughly the same amount of vegetation. Decomposition was allowed to proceed in the laboratory under monsoon conditions with a water temperature of 28.5°C. to 30.5°C. The initial stages of decomposition were quite different for each plant, but after a time the products of decomposition became more uniform, judging by the appearance and odour of the water, which became dark-brown and emitted a constant foul odour.

From time to time, samples were withdrawn for analysis; it was not found possible to make a complete analysis of the forms of nitrogen in any single case, and most attention was paid to the Tidy figures, with occasional ammonia estimations. The figures are shown in Table VII.

In the absence of sufficient data for free ammonia and albuminoid ammonia, it is difficult to say exactly what part they play, but for the present purposes the Tidy figure itself reflects very closely the course and duration of decomposition. Using this figure, *Melastoma* appears to be the least efficient plant used; it decomposed very quickly and the maximum Tidy figure is well below the maxima of the other two plants. *Eugenia* and *Eupatorium* both give very high Tidy figures, but the course of decomposition is different in each plant, as shown in Graph 1. *Eupatorium*, with its more succulent stems,

---

\*For the identification of these plants the author is indebted to Dr. W. Wight of the Botanical Department at Tocklai.



TABLE VII.

*The pollution of water by decomposing vegetation. Laboratory experiments with concentrated rotting mixtures of Melastoma, Eugenia, and Eupatorium. (Water temperature 28.5°C. to 30.5°C. All analyses in parts per million. Tidy figure—4 hours at 40°C.).*

MELASTOMA.				EUGENIA.				EUPATORIUM.			
Day.	Free ammonia.	Albuminoid ammonia.	Tidy figure.	Day.	Free ammonia.	Albuminoid ammonia.	Tidy figure.	Day.	Free ammonia.	Albuminoid ammonia.	Tidy figure.
4	Trace	1.60	76.61	5	..	..	313.25	2	..	..	750.00
6	0.10	0.96	40.59	10	..	..	452.17	4	6.15	21.79	..
7	..	..	42.71	13	..	..	634.78	7	..	..	317.95
11	..	..	29.06	18	..	..	668.42	15	..	..	269.57
14	..	..	31.45	20	0.12	6.00	..	22	..	..	244.25
19	0.0	1.60	22.26	23	..	..	724.78	36	..	..	178.18
26	..	..	13.33	30	..	..	855.65	38	8.00	9.23	..
31	..	..	5.29	38	..	..	730.97	50	..	..	142.86
				52	..	..	705.45	57	..	..	123.85
				54	6.67	12.0	..				
				66	..	..	537.50				

decomposes quickly, the maximum Tidy figure—and also high figures for free ammonia and albuminoid ammonia—being reached within a few days and quickly falling off again. *Eugenia*, which has more woody tissue, has a prolonged course of decomposition, and the Tidy figure may take a month to reach its maximum, falling off very gradually after that time.

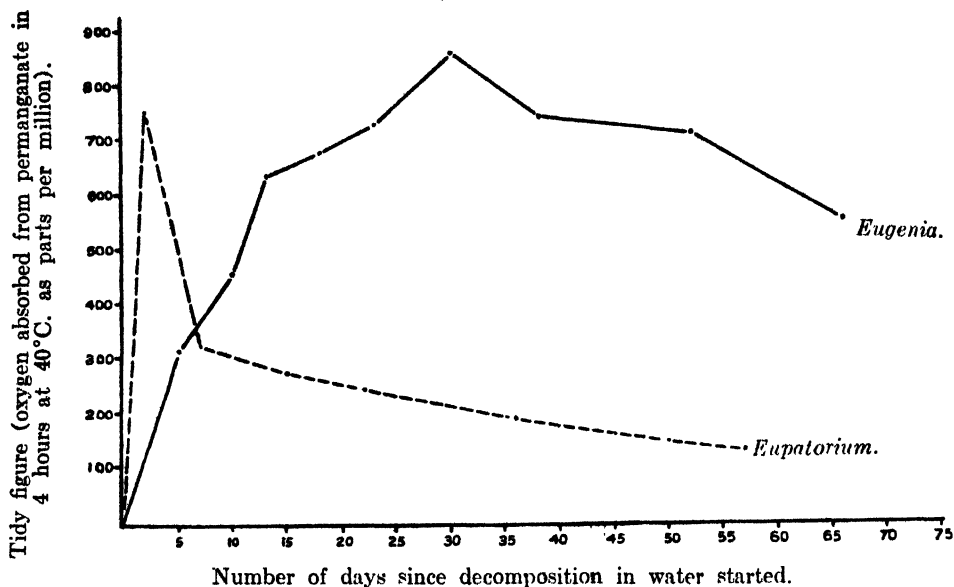
#### (2) *Course of pollution in field.*

The experiments were now extended to the field, several small brickfield borrowpits being used for this purpose. As very few anophelines of any kind were found in these pits, the chemical data alone were studied, but similar experiments involving the influence of pollution on the anophelines were carried out later under more suitable conditions. To different borrowpits were added freshly cut bushes of the three plants used above, which were all common in the brickfield area, and the course of pollution was followed by more detailed analyses than was attempted in the laboratory experiments. During this



GRAPH 1.

The course of decomposition in water of two common plants, *Eugenia* and *Eupatorium*, as indicated by the Tidy figure (oxygen absorbed from permanganate in 4 hours at 40°C.), see Table VII.



period, the amount of water in the borrowpits was subject to great fluctuations, depending on the amount of rainfall, and this obviously affected the analyses figures. The results are shown in Tables VIII, IX and X.

TABLE VIII.

Pollution of borrowpit with cut jungle. (1) *Melastoma*. Experiment started on 18/vi. All analyses in parts per million.

Day.	Free ammonia.	Albuminoid ammonia.	Tidy figure.	Nitrite.	Dissolved oxygen.	REMARKS.
0	0	0.160	2.63	0	..	Analysis before pollution. More <i>Melastoma</i> added on 20/vi.
2	0	0.320	3.94	..	..	
6	0	0.430	5.21	..	..	
9	Trace	0.790	9.32	..	..	
14	..	..	6.13	..	..	
16	0	1.200	..	0	..	Pit flooded after heavy rain.
17	..	..	5.04	..	..	
20	..	..	..	..	4.09	
22	..	..	6.71	..	..	
23	0	0.710	..	..	..	Water very low during dry spell, no smell or signs of pollution.
24	..	..	..	0	6.24	
27	..	..	..	..	..	
						Pit dried up during drought.



TABLE IX.

*Pollution of borrowpit with cut jungle. (2) Eugenia. Experiment started on 26/vi. All analyses in parts per million.*

Day.	Free ammonia.	Albuminoid ammonia.	Tidy figure.	Nitrite.	Dissolved oxygen.	REMARKS.
0	..	..	5.85	..	..	Analysis before pollution.
6	..	..	11.52	..	..	No obvious change in smell or appearance.
8	0	0.631	..	0	..	
9	..	..	4.48	..	..	Pit flooded after rain.
12	..	..	..	..	3.69	More <i>Eugenia</i> added.
14	..	..	10.44	..	..	Level of water very low.
15	0	0.412	..	0	..	
16	..	..	..	..	3.79	
19	0	2.520	16.62	..	..	Level low during drought.
26	..	..	8.61	..	..	Level up after rain.

Experiment brought to an end by continuous heavy rain and flooding.

TABLE X.

*Pollution of borrowpit with cut jungle. (3) Eupatorium. Experiment started on 5/vii. All analyses in parts per million.*

Day.	Free ammonia.	Albuminoid ammonia.	Tidy figure.	Nitrite.	Dissolved oxygen.	REMARKS.
0	0	0.196	3.87	..	..	Analysis before pollution.
3	..	..	..	..	2.78	Slight scum on surface.
5	0	0.631	7.94	..	..	
7	..	..	..	..	3.33	
10	0	1.290	7.99	..	..	Level low during drought.
17	..	..	4.86	..	..	Level up after rain, no sign of pollution.

Experiment interrupted by heavy rain with flooding for several days.

From these figures, it is seen that the course of pollution can be followed by either the albuminoid ammonia figure or the Tidy figure, or both in conjunction. In this type of vegetable pollution, the free and saline ammonia test is again shown to be quite useless as an indicator of the condition of the water, as it is never present in more than a trace even at the height of



pollution, and it can obviously play no part in rendering such water unsuitable or repellent to *Anopheles*. The complete absence of nitrite is also interesting. The dissolved oxygen figures are rather incomplete, but they are sufficient to show that the amount of oxygen may still remain high even when there is a great increase in the albuminoid ammonia and Tidy figures. For example in the experiment with *Eugenia* (Table IX), the second figure for dissolved oxygen, 3.79 mg. per litre, corresponds to a percentage saturation of 51 per cent at the temperature of sampling, and this sample was taken when the water level was low and the amount of pollution approaching a maximum. A sample taken from a nearby unpolluted borrowpit at the same time gave a concentration of oxygen of 110 per cent saturation at the temperature of sampling. Although the dissolved oxygen is lower in the polluted water, it is evidently not a figure of much value in itself as an indicator of pollution. The absence of any obvious relationship between the photo-synthetic production of oxygen and the rate of decomposition of organic matter has been pointed out by Butcher *et al.* (1937).

As far as pollution by vegetation or cut jungle is concerned, therefore, albuminoid ammonia and the Tidy figure are the two analyses which give us the best indication of the condition of the water in the breeding place, although in the event of pollution by sewage or other animal organic matter other factors would have to be taken into account.

In all these experiments, it was observed that a considerable amount of vegetation was necessary to change the appearance of the breeding place, and although the water contained an increasing amount of brown colouring matter, like peaty water, it never reached the foul-smelling offensive stage shown by concentrated mixtures in the laboratory. Furthermore, the effects of pollution did not last longer than a few weeks, decomposition probably being accelerated by the high water temperature.

In the Dooars of North Bengal, *A. minimus* larvæ are frequently found in clear, grassy-edged borrowpits during cool wet spells (Ramsay and Macdonald, 1936; Harrison and Ramsay, 1933). About 6 per cent of such pits are found to be breeding places, and the usual method of control is to pollute the water with common wayside plants, such as *Eupatorium*, lantana, tarapat, *Melastoma*, etc. (Ramsay, personal communication). In the Assam valley, such breeding places are seldom found, and it was not possible to extend the previous field experiments and find out exactly what amount or degree of pollution was sufficient to render the water unsuitable. Such an ideal field experiment, involving chemical analyses together with egg and larval collections, would do much to explain precisely how this method of control is brought about. Since it was not possible to apply these methods to *A. minimus*, a similar type of experiment was carried out on a common anopheline, *A. hyrcanus*, so that its behaviour might be compared and contrasted with that of *A. minimus* as revealed by later observations.

### (3) The effect of vegetable pollution on the breeding places of *A. hyrcanus*.

*A. hyrcanus* is found breeding in many different kinds of water collections, such as tanks, ponds, swamps, borrowpits and ricefields, particularly where abundant grass or other vegetation is present round the edges. In July, this species was found breeding continually in a small grassy-edged borrowpit (Plate XVII, fig. 5) along with small numbers of *A. barbirostris*. Routine egg



PLATE XVII.



Fig. 5. Borrowpit, breeding place of *A. hyrcanus*, polluted with rotting vegetation.



PLATE XVIII.



Fig 6. Foot-hill tea garden drains.

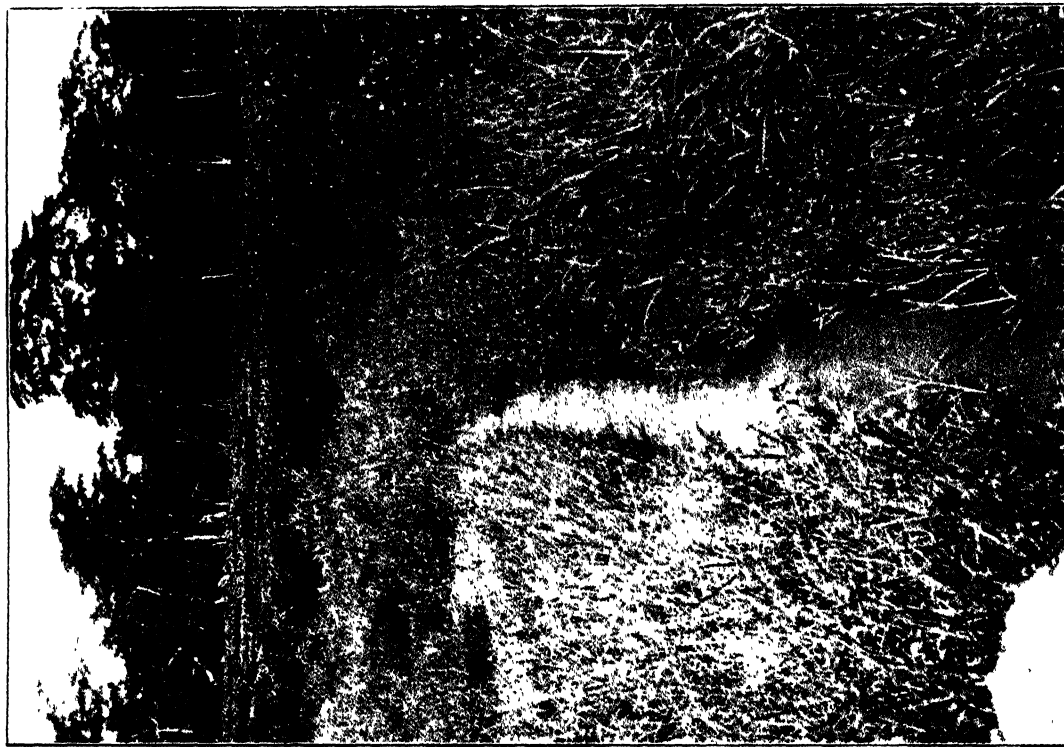


Fig 7. Foot-hill tea garden drains.



and larva collections were carried out, the eggs being collected by means of a white enamelled frying pan as described previously (Thomson, 1940a). No attempt was made to distinguish between the eggs of *A. hyrcanus* and *A. barbirostris*, but as only a few larvæ of the latter species were found it was assumed that the great majority of the eggs were those of *A. hyrcanus*. After two preliminary collections within a fortnight, a large quantity of *Eugenia*, including branches up to one inch in diameter, was introduced into the borrowpit in such a way that the grass and vegetation at the edges were left intact. Egg and larva collections were carried out about twice a week, and chemical analyses were made from time to time. At intervals, more *Eugenia* was added in order to produce the maximum possible polluting effect without altering the general appearance of the breeding place. Finally, a point was reached when any further addition of cut jungle, which was already showing above the surface of the water, would have introduced new factors such as mechanical obstruction or destruction of the vegetation at the edges. Throughout the course of the experiment, which extended over 2 months, there were no great fluctuations in the level of the water, till the pit was finally flooded by six inches of rain in one night. The results are shown in Table XI.

In this experiment, sufficient *Eugenia* was added to bring about obvious changes in the appearance and odour of the water. A brownish scum made its appearance on the surface, and this became thicker and more foul as decomposition proceeded, later being accompanied by a foul smell. The presence of a surface scum introduced an unexpected complication, as it made the detection of eggs more difficult. Despite these chemical and physical changes in the water, the results show that larvæ of all stages were regularly collected, and, what is more important, eggs were still found even at the height of pollution. The changes in composition of the water evidently had no marked injurious effect on the larvæ, and the presence of recently laid eggs affords convincing proof that the breeding place was still attractive to females of *A. hyrcanus*. The chemical figures show that free and saline ammonia remained a negligible factor during the greater part of the experiment. The albuminoid ammonia did not change very much throughout the experiment. The Tidy figure itself, however, was an accurate index of the course of pollution, and the highest figures coincided with periods when pollution, as judged by appearance and odour, was at its greatest. From the table it will also be seen that, despite the large amount of cut jungle added to the borrowpit, the maximum pollution is sustained only for a very short time.

Russell and Jacob (1939) found that pollution of casuarina-pits with casuarina slashings and other plants was only effective against all anophelines for a very short time; *A. culicifacies*, the vector species, being more susceptible to such treatment than *A. subpictus*. Although it was not possible to carry out such an experiment on *A. minimus* for reasons stated above, it is felt that many other vector species, which can breed in stagnant water, would lend themselves admirably to this kind of experiment, and that some points of technique ought, therefore, to be discussed more fully. Essentially similar types of experiment were carried out by Buxton and Hopkins (1927), in a long series of observations on the influence of different organic infusions on egg-laying in *Aedes*, but such ideal conditions are seldom realized when working with Anopheles in the field. The present experiment on *A. hyrcanus*, involving both egg counts and chemical analysis of the degree of pollution, is considered to be a further improvement on previous experimental technique. As pollution has no deterrent effect on



*The effect of vegetable pollution on the breeding place of A. hyrcanus. Grassy-edged borrowpit polluted with Eugenia. Experiment from July 11 to September 11.*

CHEMICAL ANALYSIS IN PARTS PER MILLION.				LARVÆ AND EGGS.				REMARKS.
Day.	Free ammonia.	Albuminoid ammonia.	Tidy figure.	Larvæ.			Eggs.	
				Day.	hyrcanus.	barbirostris.		
..	..	..	..	..	22	..	41	Preliminary collections before pollution. <i>Eugenia</i> added on 24/vii.
..	..	..	5.61	..	20	..	31	
..	..	..	..	..	..	..	..	
2	0.000	0.694	..	2	11	1	25	More <i>Eugenia</i> added on 27/vii.
7	0.000	0.960	8.25	7	20	1	60	No obvious signs of pollution.
9	0.000	0.640	7.27	10	..	..	25	
				13	32	3	..	Surface with slight scum.
16	0.000	0.686	9.27	17	82	9	50	No obvious signs of pollution.
20	..	..	13.36	21	41	2	65	Signs of pollution, brownish scum. More <i>Eugenia</i> added on 10/viii.
21	0.000	0.727	..	24	..	..	58	More <i>Eugenia</i> added on 14/viii.
27	Trace	0.856	16.05	27	23	6	28	Obvious pollution and foul smell. More <i>Eugenia</i> added on 22/viii.
34	Trace	0.912	17.05	34	20	3	33	Foul smell and thick brown scum. Eleven of the 33 eggs had hatched.
41	0.080	0.720	18.17	41	9	0	15	Two of the 15 eggs were empty.
49	Trace	1.000	16.13	49	21	3	28	Water packed with <i>Eugenia</i> but no foul smell. Thick scum still present.
57	0.400	0.672	12.68	..	..	..	..	Water level low.
59	..	..	..	..	..	..	..	Experiment terminated.



egg-laying in *A. hyrcanus*, a control experiment was not essential, but if the pollution had resulted in the disappearance of eggs or larvæ, or both, from the borrowpit, it would have been necessary to use another nearby breeding place as control, and find out if larvæ were still being found there and eggs still being regularly recorded. It must not be forgotten also that eggs may be difficult to find in a polluted borrowpit, not necessarily because few are being laid, but because the presence of a scum or the addition of foul suspended matter may make it increasingly difficult to detect the eggs present.

It is doubtful if this exact kind of experiment could be carried out in streams or drains or running water generally, owing to the great difficulty of finding eggs in such places, and before attempting to do so with *A. minimus*, the behaviour of this species will be studied in a series of laboratory experiments.

#### E. THE REACTIONS OF *A. MINIMUS* TO POLLUTED WATER.

##### (1) *Gravid females.*

The object of the experiments described in this section is to try and find out how *A. minimus* behaves in the laboratory when given the choice of laying its eggs in clean water, or in water polluted in different degrees with rotting vegetation. In the experiments described earlier on the course of pollution in the laboratory, dishes of concentrated rotting mixtures were used, and the highly polluted water in similar dishes was used as a stock solution in the present instance. In order to repeat each experiment two or three times and to have a graded series of polluted waters, it is necessary to have some measured standard of pollution. Simple dilution of the stock mixture is not sufficient, as its composition differs widely at different stages of decomposition. Previous experiments have shown that the albuminoid ammonia and the Tidy figure in conjunction may be used, and that of these two analyses the Tidy figure is the best single indicator of vegetable pollution. The value of the Tidy figure by itself was shown both in the laboratory experiments on the decomposition of *Eugenia*, *Eupatorium*, and *Melastoma*, and in the field experiments on *A. hyrcanus*, and it is, therefore, used in the present experiments. For the unpolluted control, freshly collected water from two or three foot-hill streams was used; this clear water had a low albuminoid ammonia figure, from 0.080 to 0.185 parts per million, and a low Tidy figure, from 2.3 to 3.2 parts per million. Clear drain water was put in one dish, and in the other the same water to which had been added sufficient of the stock rotting mixture to give a Tidy figure of 40, 20, 10, etc. Suppose the stock solution had a Tidy figure of 210 parts per million (i.e., 210 mg. per litre of oxygen absorbed from permanganate in 4 hours at 40°C.) and it was desired to have about 500 to 700 c.c. of water polluted to a Tidy figure of 40 parts per million, then 100 c.c. of the stock solution diluted with 430 c.c. of drain water would give approximately the figure required. Assuming that the water used for dilution has a Tidy figure of 0, then 100 c.c. stock solution contains oxidizable organic matter = 21 mg. oxygen.

∴ 530 c.c. diluted solution contains organic matter = 21 mg. oxygen

∴ 1,000 c.c. of same solution would contain organic matter equivalent to

$\frac{21 \times 1,000}{530}$ , i.e., 39.6 mg. Therefore, the Tidy figure is approxi-

mately 40 mg. per litre, or 40 parts per million.



As the drain water used for dilution had a certain amount of dissolved organic matter, the female mosquito will really be exposed to a choice of clear water with a low Tidy figure which we may call  $a$ , and polluted water with a figure of  $a + 40$ ,  $a + 20$ , etc.

Although the conditions in this exact laboratory experiment are artificial, it is reasonable to suppose that the mosquito might be faced with very much the same choice in the field if its natural breeding place was polluted with cut vegetation. As *Eupatorium* seemed to be the most likely plant for polluting natural breeding places of *A. minimus*, it was used as the polluting agent in all these experiments.

Gravid females were first exposed to a wide choice of pollution, with a high Tidy figure in the polluted dish. Then the range was narrowed till the difference was no longer appreciated by the mosquito. All the experiments were carried out in a  $2 \times 2 \times 2$  ft. cage, which was darkened with sheets of paper at night when eggs were being laid, so as to avoid any complications due to unequal illumination.

The results are set out in Table XII.

TABLE XII.

*Eggs laid by A. minimus when offered a choice of clean drain water and water polluted to different degrees by Eupatorium.*

I. CHOICE OF DRAIN WATER AND WATER POLLUTED TO TIDY FIGURE OF 40 PARTS PER MILLION.

Date.	Drain water.	Polluted water.	
8/ix	1,418	755	Out of 4,615 eggs, 81.2 per cent were laid in drain water.
10/ix	904	102	
11/ix	1,427	9	
Total	3,749	866	

II. CHOICE OF DRAIN WATER AND WATER POLLUTED TO TIDY FIGURE OF 20 PARTS PER MILLION.

Date.	Drain water.	Polluted water.	
14/ix	659	62	Out of 721 eggs, 91.4 per cent were laid in drain water.



TABLE XII—*concl'd.*

## III. CHOICE OF DRAIN WATER AND WATER POLLUTED TO TIDY FIGURE OF 8 PARTS PER MILLION.

Date.	Drain water.	Polluted water.	Out of 2,028 eggs, 70·7 per cent were laid in drain water.
16/ix	966	364	
17/ix	469	229	
Total	1,435	593	

## IV. CHOICE OF DRAIN WATER AND WATER POLLUTED TO TIDY FIGURE OF 5 PARTS PER MILLION.

Date.	Drain water.	Polluted water.	Out of 4,973 eggs, 71·9 per cent were laid in drain water.
20/ix	464	444	
21/ix	907	424	
12/x	2,204	530	
Total	3,575	1,398	

## V. CHOICE OF DRAIN WATER AND WATER POLLUTED TO TIDY FIGURE OF 3 PARTS PER MILLION.

Date.	Drain water.	Polluted water.	Out of 3,730 eggs, 45·4 per cent were laid in drain water.
13/x	901	558	
18/x	795	1,476	
Total	1,696	2,034	

When we consider that the Tidy figure is only an estimate of the organic matter, giving no indication of its quality, the results are surprisingly consistent. They show that, even when the Tidy figure of the polluted water is as low as 5 parts per million there is still a marked preference for the clean water, over 70 per cent of the eggs being laid there. When the available choice is still further reduced, behaviour becomes erratic, and females show no regular preference when given a choice of laying in drain water and the same polluted to a Tidy figure of 3 parts per million.



Since a Tidy figure of 5 parts per million seems to be the lowest pollution of this kind distinctly perceived by *A. minimus*, some more details of the conditions in these particular experiments might be instructive. In the first two experiments, the polluting agent came from a dish in which *Eupatorium* had been rotting for 4 weeks, and in the third experiment, it came from another dish of *Eupatorium* which had been rotting for the same time. This second dish decomposed more slowly, and had a Tidy figure of about 492 at the time of the experiment, and to produce a dilution with a Tidy figure of 5 parts per million this stock solution had to be diluted by 1 part with 95 parts of drain water. At this high dilution it was just possible, with a little practice, to distinguish the faint odour of the polluted water as compared with the clean drain water.

In all these experiments, the eggs laid in polluted water tended to keep together in clumps in the open surface, while in the dish of clean drain water they tended to separate and spread to the edges. This is probably due to the fact that the surface tension of water is lowered by organic substances in solution, and even with the high dilutions used this phenomenon was still seen.

Another observation of interest is that the later products of decomposition are not so effective in repelling the female mosquitoes. Four experiments were carried out with water from dishes in which *Eugenia* and *Eupatorium* had been rotting for 2 months or more. When these old stock solutions were diluted with drain water to give a Tidy figure of 40 parts per million, females of *A. minimus* failed to distinguish the polluted water from the clean water, and their behaviour was quite erratic. In four experiments involving both *Eugenia* and *Eupatorium*, out of 6,711 eggs 48.4 per cent were laid in drain water. Despite the high Tidy figure of these old stock solutions, it is evident that the nature of the organic matter is quite different from that produced during the first 4 or 5 weeks of decomposition. It is probable that these later products of decomposition, which were allowed to accumulate under artificial conditions in the laboratory, would, under field conditions, be quickly oxidized or broken down, and no longer affect the Tidy figure.

The great sensitivity of *A. minimus* females to the earlier products of vegetable decomposition is in striking contrast to the behaviour of *A. hyrcanus*. While *A. hyrcanus* continued to lay eggs in water polluted to a Tidy figure of approximately 13 parts per million (i.e., 18.17 to 5.61, see Table XI), *A. minimus* still showed a significant avoidance of water polluted to a Tidy figure of 5 parts per million. It is evident that this behaviour must play a large part in control by vegetable pollution or herbage packing, and its full significance will be discussed later in the light of further experiments.

It is interesting to compare the behaviour of *A. minimus* with that of a large culicine, *Armigeres (Armigeres) kuchingensis* var. *shillongensis*\* which was found breeding in the dishes of concentrated rotting plants, *Eupatorium*, *Eugenia*, and *Melastoma*, and was regularly attracted to the foul-smelling water after the cut plants had reached a certain stage of decomposition. The degree of pollution of these concentrated mixtures, as shown by the Tidy figure and the albuminoid ammonia, was at least 100 times greater than that avoided by the gravid females of *A. minimus*.

---

\*For the identification of these culicines, the author is indebted to Dr. I. M. Puri, of the Malaria Institute of India.



(2) *Larvæ*.

A series of experiments was carried out to see if *A. minimus* larvæ were sensitive to water polluted with decaying vegetation, and to try and find out what degree of pollution would prevent the larvæ from reaching full growth and pupating. Batches of 100 or 200 newly hatched larvæ were put in large dishes with water containing different amounts of the stock mixtures of rotting plants; some grass from the natural breeding was also supplied. A preliminary series of experiments showed that controls placed in clean water gave erratic results and often exhibited a heavy mortality, and, therefore, no attempt was made to compare the growth or mortality in different strengths of pollution.

In one dish contaminated with rotting *Eupatorium* and having an initial Tidy figure of 68 parts per million, out of 100 newly hatched larvæ, 70 eventually pupated and emerged within 3 weeks. During the course of the experiment the strength of pollution gradually fell off, and 12 days after the experiment started the Tidy figure had fallen to 30 parts per million.

In a second, more highly polluted dish the initial composition was Tidy figure 160 parts per million, albuminoid ammonia 10 parts per million, free and saline ammonia 10 parts per million.

Out of 100 newly hatched larvæ, 45 finally pupated and emerged. As before the strength of pollution gradually decreased, and 3 weeks after the experiment started the Tidy figure was 70.

It appears from these figures that *A. minimus* larvæ, although they normally live in clean, unpolluted water, can tolerate a high degree of pollution of this kind. The strength of pollution in both experiments is much higher than could be obtained in the field, and shows that pollution of the breeding place is unlikely to produce its controlling effects by killing the larvæ. Manson (1939), however, has shown that there are certain plants such as *Duranta*, *Gardenia*, and *Zanthoxylum*, which are larvicidal in aqueous solution up to certain dilutions such as 1: 50 and 1: 100. In those cases, the lethal effect has been traced to definite substances, for example saponins, which are present in the living plant and are not decomposition products.

From the present experiments on *A. minimus*, it seems that in most cases the products of vegetable pollution are not noticeably toxic to larvæ, and that such a high figure for free and saline ammonia as 10 parts per million does not signify that the water is unsuitable for development.

Puntoni (1934) found that larvæ of *A. maculipennis* var. *labranchiæ* developed as readily in undiluted sewage as in spring water, and Russell and Mohan (1940) reared larvæ of *A. stephensi* in sullage water containing up to 50 parts per million of ammonia nitrogen and 6 parts per million of albuminoid nitrogen.

The most important fact which emerges is the wide difference between the reactions of the gravid females and of the larvæ of *A. minimus*. When exposed to the same vegetable pollution, larvæ can live and grow successfully in water with an amount of pollution (as shown by the Tidy figure) over 30 times greater than that avoided by the female when laying its eggs. The sensitivity of the female to pollution is so much greater than that of the larva, that it must be the deciding factor in most cases of control by pollution or contamination, and this conclusion is fully supported by further experiments which will be described later in this paper.



F. THE POLLUTION OF *A. MINIMUS* BREEDING PLACES WITH  
ROTTING VEGETATION.(1) *Herbage packing.*

The method of polluting clear running water in streams and open drains by closely packing the drain with cut jungle and green vegetation was first elaborated by Williamson (1935), and since that time it has been applied successfully in India by Senior White (1936) and Covell and Harbhagwan (1939). In the control of *A. minimus*, vegetable pollution has been found successful in borrowpits, but the method has not been extended to running water, although Ramsay and Macdonald (*loc. cit.*) and Ramsay (personal communication) have shown that short stretches of irrigation channels can be controlled by periodic contamination with cart loads of cow-dung.

It was decided to carry out some critical controlled experiments on the effect of herbage packing on a breeding place of *A. minimus*. The idea was to pack a length of drain with cut jungle, principally *Eupatorium*, follow the course of pollution at different distances down the stream by means of chemical analyses, and make routine searches for larvæ and, if possible, for eggs. Unfortunately, the season was an extremely poor one, and the opportunities of carrying out this simple, but crucial, experiment never once arose. When it was realized that there was no further hope of doing this, an attempt was made to study the chemical aspects alone. In October, after the end of the rainy season, 185 feet of a small foot-hill drain of the type shown in Plate XVIII, fig. 6, was tightly packed with cut stems of *Eupatorium*. The experiment was a failure, as the plants failed to rot and pollute the water; the failure is instructive, however, because experiments carried out in the laboratory showed that at this time of the year *Eupatorium* very quickly loses its powers of rotting in water.

*Eupatorium* packed in a dish of water at the beginning of September rotted slowly and the water eventually became black and foul-smelling, but the same treatment at the end of September produced no signs of decomposition after several weeks. The fact that some plants may become unsuitable for herbage packing in the dry weather has already been pointed out by Senior White (1936), but even then this sudden change in the nature of *Eupatorium* was quite unexpected, and shows that such a method of control might have its limitations.

Despite the failure of these field experiments to prove anything definite, the gap can largely be filled by similar experiments carried out on the closely related *A. fluviatilis* (Covell and Harbhagwan, *loc. cit.*) in which herbage packing of ricefield drainage channels was found to be a very successful control measure against that mosquito.

In the absence of the necessary field experiments, it is impossible to give an opinion on the possibilities of this method of control for *A. minimus*, but there is another type of vegetable pollution the use of which has already been established and which will now be discussed in detail.

(2) *Pollution of tea garden drains with factory effluent.*

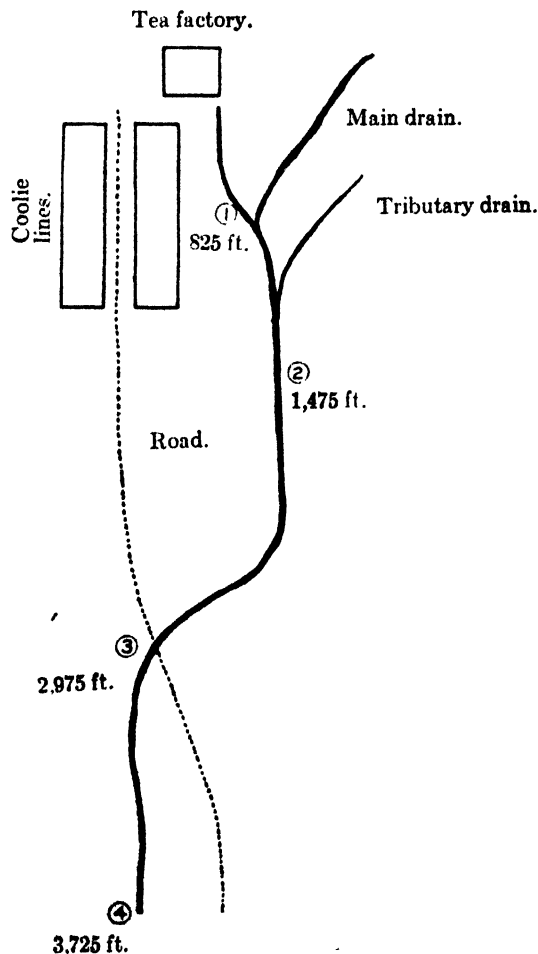
*Field experiments.*—In one foot-hill tea garden, it was found that a long stretch of grassy-edged drain—of the type shown in Plate XVIII, fig. 7—was kept free of *A. minimus* for several months of the year by running the factory effluent into the drain. The malaria surveyor reported that throughout the



period the factory is in operation, i.e., roughly from the end of March till the end of November, no larvæ are found, although they may occur after this season. The effluent is principally composed of washings from the tea rollers, and is, therefore, almost entirely vegetable matter; there is a constant trickle into the drain, and it imparts a particularly foul smell to a long stretch of water.

### Text-figure.

Showing garden drain polluted by tea factory effluent, tributary drain arising from seepages, and position of four sampling stations.



A detailed experimental investigation of this (accidental) method of control was carried out on the same lines as before, by making a chemical study of the drains affected and finding out how *A. minimus* reacted to this kind of pollution. A rough plan of the different drains is shown in the text-figure. For the water analyses, four collecting stations were selected; the first station, 825 feet from



the factory, was for sampling the undiluted effluent just before it entered the main drain. Between the second and the third station the drain was joined by another. The second, third, and fourth stations were 1,475, 2,975, and 3,725 feet from the factory respectively. Shortly below the fourth station, the drain discharged into a river. In the intervals of drought between heavy showers, the water in the whole length of the drain was blackish and evil-smelling, and there was very little dilution from the small tributary drains. At this time, when there was only a very slow flow, the water at Station 3 was blacker and more offensive than at the other three stations. The composition of samples taken under these conditions is shown in Table XIII, Experiments A and B.

TABLE XIII.

*Pollution of a garden drain by tea factory effluent. Record of chemical analysis during October and November.*

(All analyses in parts per million.)

Expt.	Date.	Sampling station.	Free ammonia.	Albuminoid ammonia.	Tidy figure.	Nitrites.	Dissolved oxygen.
A.	14/x	I	5.710	4.430	73.51	0	0.00
		II	..	..	53.15	0	0.00
		III	10.000	5.150	105.77	0	0.36
		IV	..	..	77.12	0	0.35
B.	21/x	I	..	..	80.26	..	..
		II	2.500	3.360	75.44	..	..
		III	..	..	125.44	..	..
		IV	4.350	3.360	102.63	..	..
C.	25/x	I	5.330	4.700	115.93	Trace	0.00
		II	0.870	1.250	13.45	0	3.79
		III	0.440	0.720	15.22	0	3.39
		IV	0.130	0.910	14.87	0	4.83
D.	7/xi	I	3.810	1.330	17.12	Trace	0.70
		II	3.810	1.660	21.17	0	4.51
		III	7.680	2.850	85.59	0	1.02
		IV	4.540	2.780	51.80	0	6.04

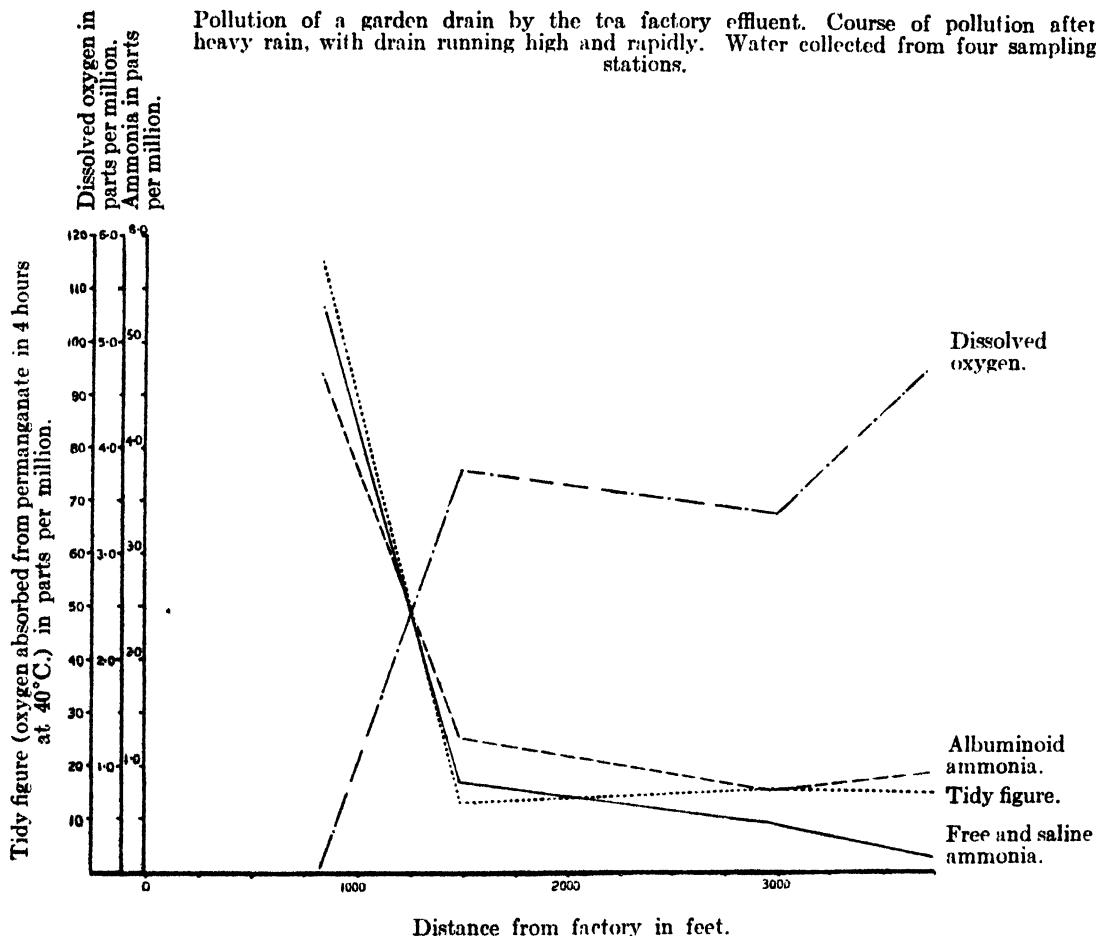
From these figures it is seen that in all sections of the drain the figures for free and saline ammonia, albuminoid ammonia, and the Tidy figure indicate a



very high degree of pollution, not unlike that of the concentrated rotting mixtures previously used as stock solutions in the laboratory. Instead of finding that the degree of pollution falls off further down the drain, it is seen that the third station, where the water was blackest and most offensive had the highest figures in both experiments. Mr. S. F. Benton, the bacteriologist of the Tea Research station, has kindly informed me that the biological processes involved are probably as follows: the *aërogenes* group of bacteria which are

GRAPH 2.

Pollution of a garden drain by the tea factory effluent. Course of pollution after heavy rain, with drain running high and rapidly. Water collected from four sampling stations.



responsible for the black colour and foul smell do not come from the factory but enter the drain from outside. In the lower half of the drain, decomposition of the proteins has been proceeding, and has resulted in the production of amino-acids, amines, and free ammonia; this produces an alkaline medium, in which oxidation and condensation of tannins produce the characteristic black colour.

This type of vegetable pollution differs most noticeably from those of previous field experiments in the high figure for free and saline ammonia, up



to 10 parts per million. Nitrites are absent, and dissolved oxygen is only present in small quantities.

This series of analyses was repeated after 2 and 3 inches of rain had fallen, and had resulted in the whole drain running high and rapidly. The effluent at Station 1 was increased by seepage water, and there was a steady flow of clean water, equal in bulk to the effluent, coming in from the tributary drains. Although the water had lost its black colour, there was still a perceptible smell throughout the length of the drain. The results are shown in Table XIII, Experiment C, and in Graph 2.

It will be seen that at Station 2 the Tidy figure and both ammonia figures have fallen off rapidly, owing to the diluting effects of the drain water and that there is a corresponding sharp rise in the amount of dissolved oxygen. From this point downstream, however, the figures remain fairly steady and the degree of pollution at Station 4, nearly three-quarters of a mile from the factory, is still high, with a Tidy figure of 15 and albuminoid ammonia figure of 1 part per million. Despite the high concentration of dissolved oxygen at this point, there is little evidence of self-purification, except in the free ammonia figures. On this day nitrite was present in the crude effluent at Station 1, but absent from lower down the drain.

A fourth series of samples were tested about a fortnight after the heavy rain. The drain was flowing sluggishly, and the figures (Table XIII, Experiment D) show a reversal to conditions experienced before the rain (Table XIII, Experiments A and B) with the highest degree of pollution at Station 3.

Before these figures can be fully appreciated, it will be necessary to find out how *A. minimus* reacts to this type of organic pollution.

*Reactions of gravid females.*—To find out how gravid females of *A. minimus* react when given the choice of laying eggs in clean water and water polluted to different degrees with factory effluent, the methods used were exactly the same as those described for pollution by rotting *Eupatorium*. The heavily polluted water from Station 3 was analysed and then diluted in such a way as to give a Tidy figure of 20, 10 and 5 parts per million. Such dishes of water with a known degree of pollution were prepared in the afternoon, exposed to the mosquitoes overnight, and then discarded next morning after the eggs had been collected and counted. Check analyses after the experiment showed that there was no great change in composition overnight; for example, a dish of water polluted to a Tidy figure of 5 parts per million gave a figure of 4.6 the next day. The control in these experiments was clean, unpolluted drain water as before. The results are shown in Table XIV.

These results, which should be compared with those of Table XII, show that pollutions giving Tidy figures of 20 and 10 are distinctly perceived by the female mosquito, and there is a consistent preference for laying eggs in the clean drain water. When the effluent is diluted to give a Tidy figure of 5 parts per million, there is still a slight preference for the clean water, but the behaviour is becoming more erratic, more eggs being laid in the polluted water in two of the five experiments. Owing to the strong smell of the effluent, it was thought that the mosquitoes would be sensitive to even higher dilutions than those found for rotting *Eupatorium*. Here again, however, dilutions giving a Tidy figure of 5 parts per million are near the limit perceived by the mosquitoes. At this dilution, the smell was much stronger than the corresponding dilution of *Eupatorium*, but the intensity of the reaction was not so great, 72 per cent



TABLE XIV.

*Eggs laid by A. minimus when offered a choice of clean drain water and water polluted to different degrees with tea factory effluent.*

I. CHOICE OF DRAIN WATER AND WATER POLLUTED TO TIDY FIGURE OF 20 PARTS PER MILLION.

Date.	Drain water.	Polluted water.	
8/xi	1,292	0	. Out of 3,551 eggs, 81.7 per cent were laid in drain water.
9/xi	591	199	
10/xi	1,019	450	
Total	2,902	649	

II. CHOICE OF DRAIN WATER AND WATER POLLUTED TO TIDY FIGURE OF 10 PARTS PER MILLION.

Date.	Drain water.	Polluted water.	
26/x	1,071	374	Out of 3,102 eggs, 76.2 per cent were laid in drain water.
27/x	425	179	
29/x	869	184	
Total	2,365	737	

III. CHOICE OF DRAIN WATER AND WATER POLLUTED TO TIDY FIGURE OF 5 PARTS PER MILLION.

Date.	Drain water.	Polluted water.	
19/x	895	182	Out of 6,612 eggs, 60.6 per cent were laid in drain water.
22/x	687	898	
23/x	353	0	
24/x	1,309	686	
25/x	763	839	
Total	4,007	2,605	



of the eggs being laid in clean water in the *Eupatorium* experiments, as compared with 61 per cent in the factory effluent experiments. From this it would appear that the avoidance of polluted water is not just a simple olfactory response. When we consider the two very different types of vegetable pollution involved, the general trend of the figures in Tables XII and XIV is remarkably similar, and suggests very strongly that the Tidy figure itself is a fairly good estimate of those products of vegetable decomposition which are repellent to the gravid female.

If we refer to Table XIII, it is seen that the Tidy figure of the drain water polluted by factory effluent usually remains high throughout the drain, and that even when the drain is running high after heavy rain this figure never falls as low as 10 parts per million, a degree of pollution distinctly appreciated by *A. minimus*. In the short periods of drought between heavy showers, the Tidy figure of the polluted drain frequently exceeds 100, with corresponding high figures for free ammonia and albuminoid ammonia, and at such times there would be sufficient effluent to contaminate successfully two or three similar drains.

*Reactions of larvæ*.—A few experiments were carried out to see whether the factory effluent had any lethal effect on *A. minimus* larvæ. Using the same methods as before, it was found possible to rear larvæ from the egg to the adult stage in undiluted drain water from Station 3. The initial composition of the water in different experiments was :

	Tidy figure.	Free ammonia.	Albuminoid ammonia.	
(1)	105	10.0	5.2	} parts per million.
(2)	125	..	..	
(3)	72	4.0	4.0	

Out of 100 newly hatched larvæ in each case, four eventually pupated in the first experiment, seven in the second, and 24 in the third. Owing to the difficulty, previously described, of carrying out controlled experiments on the growth and mortality of *A. minimus* larvæ, there are no data with which we can compare these results; nevertheless, the important fact emerges that complete growth and development of larvæ can take place in the most heavily polluted drain water, and that the reactions of the larvæ alone afford no evidence to explain how control is brought about.

## II. SILT.

### INTRODUCTION.

The turbidity or amount of silt in *Anopheles* breeding places is a factor which has attracted a great deal of attention, particularly with regard to rivers and streams and running water in general. Reports about the influence and larvicidal properties of silt are rather contradictory, however, and it is obvious that further investigation is needed on this subject. Ramsay (1930) has shown that silty water is usually unfavourable for *A. minimus* breeding, while Manson (1936) found no definite relation between the turbidity of river water and the presence or absence of *A. minimus* larvæ, although there was a close correlation between silt and variations in water velocity. De Jesus (1937) usually found *A. minimus* var. *flavirostris* in clean water with a turbidity of 0 to 20 parts per



million, but they were also found in the same breeding place when the turbidity was temporarily increased to 400 parts per million.

From observations on anopheline breeding places in general, it soon becomes apparent that there is no correlation between turbidity and water movement; small pools of stagnant water may remain silty for weeks, while the water in a nearby river continues to run clear. Freshly excavated tanks and borrowpits may retain a large amount of silt in suspension despite the absence of water movement, and in such cases there is a closer relation between the turbidity and the absence of vegetation at the edges. If we confine our attention to a particular stream or river, however, there is a more obvious correlation between siltiness and water velocity, or rather change in water velocity.

If we assume for the moment that silty water is unsuitable for *A. minimus* breeding, then there are two distinct ways in which this might be brought about: (a) when the female mosquito is looking for a place to lay its eggs it might be repelled by silty water, or (b) a high concentration of silt in the water might kill the larvæ, or prevent their growth. Field and laboratory experiments were, therefore, carried out to test these two main alternatives.

#### MEASUREMENT OF TURBIDITY.

For this purpose, two stock suspensions of fuller's earth were made up. The first contained 1 gramme in a litre of water, i.e., 100 parts per 100,000, while the second contained 10 grammes in a litre of water, i.e., 1,000 parts per 100,000. The samples of silty water were compared with the standard in Nessler cylinders, different dilutions of the standard being compared with the sample till a match was obtained.

#### FIELD EXPERIMENTS.

In a previous communication (Thomson, 1940b) it was shown that *A. minimus*, which usually oviposits in the thick grassy edge of streams or drains, would regularly lay eggs in heavily shaded, artificial, side pockets of still water, completely devoid of vegetation, and that it continued to do so even when these pockets were separated from the main stream and converted into little pits with bare edges. Although the drain was running clear, the water in these pits remained constantly silty, and this siltiness increased as the level of the water in the adjacent drain gradually fell. Despite this fact, eggs of *A. minimus* were regularly recovered from these pits, and during the period 31/x to 14/xi, 247 eggs were collected from two pits, A and B, as previously recorded. The water in both pits remained silty all the time; on comparison with the fuller's earth standard, one pit had a silt content of 190 parts per 100,000 on 10/xi, and 266 parts per 100,000 on 14/xi. As there was no vegetation in these pits, and no deposit of decaying mud on the bottom, the water remained unpolluted, with a low albuminoid ammonia and Tidy figure, as shown by later analysis in similar pits.

From these experiments, we can conclude, therefore, that siltiness in itself is not repellent to the ovipositing female under natural conditions, even when it is offered the alternative of laying its eggs in clear water.

On 7/xi many young *A. minimus* larvæ were found in these pits, and on each occasion when eggs were collected larvæ were also collected and counted and replaced in their original pit. The development of larvæ in these silty pits is shown in Table XV. By 24/xi the pits were rapidly drying up, and



larvæ found on that day were removed to silty water in the laboratory, where some of them completed their development. These experiments show that, despite the high silt content of the water, many larvæ grew rapidly to full size, and in a few cases pupated and emerged successfully. There is no indication here of any larvicidal or lethal effect of silt.

TABLE XV.

*Growth of A. minimus larvæ in silty pools. Siltiness in parts per 100,000 of fuller's earth suspension.*

Date.	Pit A.	Pit B.	Eggs A and B.	Silt.	REMARKS.
7/xi	55	..	32	..	Mostly very young larvæ.
10/xi	65	65	51	190	All first and second stage larvæ.
14/xi	95	50	33	266	Four larvæ full grown.
18/xi	58	38	0	308	Fourteen larvæ full grown.
21/xi	28	17	0	267	Fourteen larvæ full grown, one pupa.
24/xi	20	..	..	..	Nine full-grown larvæ, four pupated in laboratory 24-29/xi, and all emerged 28/xi-1/xii.

## LABORATORY EXPERIMENTS.

Using the same soil from these pits, a series of laboratory experiments was carried out to find if *A. minimus* larvæ were affected by an even higher amount of silt in the water. Owing to the nature of this soil, the fine silt remained in suspension for a long time without settling, and a high degree of turbidity could be maintained by stirring the dishes of water two or three times each day. Two hundred newly hatched larvæ were put in each of four dishes, which varied in turbidity from very silty to practically clear. In the four dishes, the mean silt content was equivalent to 1,120, 490, 140, and 8 parts per 100,000 respectively of fuller's earth suspension, although there was a considerable range of variation within each dish. The mortality in all dishes was very high, but the very silty water was not particularly unfavourable for larvæ. After one week, the number of larvæ in each dish was 113, 97, 76, and 112 respectively, and after 2 weeks it was 26, 27, 27, and 36. In the siltiest dish, a few larvæ were still alive after 5 weeks.

In all these field and laboratory observations, there is no evidence that silt in itself is either repellent to the gravid female or noticeably larvicidal. When a river comes down in flood, the great increase in turbidity is so marked that the corresponding changes in other characters of the breeding place are apt to be overlooked. Should the river at this stage become unsuitable for anopheline breeding, it would be quite unjustifiable to attribute the result to increased siltiness alone, without taking into account the simultaneous increase in current velocity at the edges where breeding takes place, and the great change in composition which the river water undergoes at this time. As far as *A. minimus* is concerned, the great sensitivity to water movement (Thomson, 1940b) would



afford a more reasonable explanation of the natural control usually attributed to the increased amount of silt, although at the same time this does not necessarily dismiss the possibility that, with some other species of *Anopheles*, silt itself might have an adverse effect.

### DISCUSSION.

The difference in composition between clear running water regularly used by *A. minimus* and the stagnant water of tanks, borrowpits, and ricefields, with regard to the organic matter estimated by the albuminoid ammonia and the Tidy figure, is so striking that it seemed at first to offer a reasonable solution to the ultimate determination of the breeding place. In view of the negative results obtained with gravid females and larvæ, however, this idea is no longer tenable, the purity of the water being apparently a character closely associated with clear, running water and one without any special significance in itself. This finding also serves to show that the discovery of a measurable difference in character between two different types of breeding place cannot be considered of any real significance until it has been tested by experimental methods.

The observation that *A. minimus* cannot distinguish the water of its normal breeding place from other kinds of natural water, despite the fairly constant difference in composition, closes several other lines of enquiry. It shows that there is nothing intrinsically attractive about the water itself, either chemically or biologically, automatically eliminating the possibility of algæ or plankton playing any major part in the selection of the breeding place.

Although *A. minimus* is not affected by the amount or type of organic matter encountered in natural waters, it is very sensitive to recent pollution by decaying vegetation, and the behaviour of the female in this respect throws a great deal of light on control by herbage packing or contamination of the breeding place. At this stage, it is impossible to say exactly what substance, or combination of substances, it is that repels the female mosquito when it is going to lay its eggs, but the fact that the Tidy figure itself can be used as an index, indicates that we are really dealing with some organic product of pollution and not with some uncontrolled secondary factor such as surface tension, bacteria, odour, etc.

The oxidizable organic matter in water is usually estimated by acid permanganate, but Butcher *et al.* (1937), in their work on the River Tees, have found that alkaline permanganate gives a more complete oxidation, particularly with those complex products of decomposition which are only partially oxidized by acid permanganate. These authors suggest that the ratio of the amount of oxidation with alkaline permanganate to that with acid permanganate would give a better indication of the stage of decomposition, and this suggestion might be applied with advantage to future work on *Anopheles*. A further elaboration of these chemical methods would be to investigate the *quality* of the 'dissolved organic matter' by estimating the principal constituents such as carbohydrate, organic acid, or the various forms of nitrogen, such as amino-, non-amino-, and peptide-nitrogen which have been studied by Domogalla *et al.* (1925) and Peterson *et al.* (1925) in lake water.

The absence of free and saline ammonia from most of the anopheline breeding places tested is rather surprising, and it also seems to play little part in the changes produced by polluting the water with vegetation. But naturally



these conclusions would not apply to pollution by sewage or animal organic matter, in which free ammonia plays such a prominent part.

The experiments on the oviposition of *A. minimus* and *A. hyrcanus*, although they were not carried out in similar circumstances, show that the different sensitivity of different species of *Anopheles* to pollution is almost certainly determined by the behaviour of the gravid female and not by the reactions of the larvæ; and it is this behaviour which decides whether or not control by pollution of the breeding place will be successful.

In a previous communication (Thomson, 1940a), it was shown that control of *A. minimus* by 'shade' was not due to the actual shade or absence of light, but to secondary modifications produced in the breeding place. It appears that silt occupies a somewhat similar position, the natural control often associated with the appearance of silty water being due, not to the silt itself, but to the simultaneous change in some other character, most likely water velocity, which accompanies or causes the increased turbidity.

### SUMMARY.

(1) The composition of the water in the breeding place of *A. minimus* was compared with that of water collections in which this species seldom breeds.

(2) A perennial river, the principal cold weather breeding place, was compared with fresh-water tanks, while monsoon breeding places such as tea garden drains were compared with stagnant rain-filled ricefields.

(3) There was no constant difference between the dissolved oxygen content of river water and of tank water, either by day or at night.

(4) Free and saline ammonia was seldom present in more than a trace in different anopheline breeding places, and this test was, therefore, useless for distinguishing different kinds of water.

(5) The albuminoid ammonia and the Tidy figure (oxygen absorbed from permanganate), which gave an estimate of the organic matter and the degree of pollution, were found to be the most valuable analyses for indicating the suitability of the breeding place. Both these figures were lowest in the type of water regularly selected by *A. minimus*.

(6) The degree of pollution, as estimated by a convenient formula, 
$$\frac{(\text{albuminoid ammonia} \times 100) + (\text{Tidy figure} \times 10)}{2}$$
 was 3.7 times greater

in tanks than in river water, while that of ricefields was three times greater than that of garden drains.

(7) When a river comes down in flood, there is a great increase in the degree of pollution, and there is no longer a constant difference between river and tank water.

(8) Gravid females fail to distinguish between water from the normal breeding place and water in which they do not usually breed, despite the difference in composition.

(9) The difference in composition has no marked effect on the growth and mortality of larvæ.

(10) A chemical study of the pollution of water by cut jungle or decaying vegetation was carried out in the laboratory and in the field.



(11) The course and degree of pollution can best be followed by the test for albuminoid ammonia and the Tidy figure; of these two, the Tidy figure is the best single indicator of vegetable pollution.

(12) In such polluted breeding places, free and saline ammonia are usually absent and can play no part in rendering the water unsuitable.

(13) In a breeding place of *A. hyrcanus*, vegetable pollution alone fails to make the water unsuitable, eggs still being laid at the height of pollution.

(14) Laboratory experiments showed that *A. minimus* is very sensitive to pollution by cut vegetation, gravid females being able to distinguish high dilutions of rotting vegetation.

(15) Larvæ, on the other hand, can develop successfully in water with a degree of pollution over 30 times greater than that avoided by the gravid female.

(16) The sensitivity of the gravid female must be the deciding factor in control by vegetable pollution, or herbage packing.

(17) These findings are supported by a detailed study of pollution of tea garden drains by factory effluent.

(18) Field experiments show that *A. minimus* will still lay eggs in very silty water.

(19) In such silty water, successful development and growth of larvæ can take place.

(20) Laboratory experiments support these findings, and show that silt, in itself, has no marked lethal or larvicidal effect on *A. minimus* larvæ.

(21) In rivers, the natural controlling effects usually attributed to silt are more likely to be due to the simultaneous increase in current velocity.

## REFERENCES.

- ALSTERBERG, G. (1925) .. *Bioc. Zett.*, **159**, pp. 36-47. Also *Chem. Abst.*, **20**, p. 290.
- BEATTIE, M. V. F. (1930) .. *J. Ecol.*, **18**, 1, pp. 67-80.
- Idem* (1932) .. *Bull. Ent. Res.*, **23**, pp. 477-496.
- BRANDT, H. J. (1937) .. *Gesundh. Ing.*, **60**, pp. 557-559. Also *Chem. Abst.*, **32**, p. 2657 (1937).
- BUTCHER, R. W., LONGWELL, J. and PENTELOW, F. T. K. (1937). Survey of the River Tees, Pt. III, *Dep. Sci. Ind. Res. Water Pollution Research.*
- BUTCHER, W. R., PENTELOW, F. T. K., and WOODLEY, J. W. A. (1927a). *Bioc. Jour.*, **21**, 2, pp. 945-957.
- Idem* (1927b). *Ibid.*, **21**, 3, pp. 1423-1435.
- BUXTON, P. A., and HOPKINS, G. H. E. (1927). Researches in Polynesia and Melanesia. Parts I to IV. 260 pp. with 12 plates. London School of Hygiene and Tropical Medicine, London.
- COVELL, G., and HARBHAGWAN (1939). *J. Mal. Inst. Ind.*, **2**, 4, pp. 341-377.
- DOMOGALLA, B. P., JUDAY, C., and PETERSON, W. H. (1925). *Jour. Biol. Chem.*, **63**, pp. 269-285.
- GANAPATI, S. V. (1940) .. *Proc. Nat. Inst. Sci. Ind.*, **6**, 2, pp. 237-300.
- HACKER, H. P. (1923) .. Ann. Report of the Malaria Bureau, F. M. S. 1923.
- HACKETT, L. W., RUSSELL, P. F., SCHARFF, J. W., and SENIOR WHITE, R. (1938). *Bull. Hlth. Org. L. of N.*, **7**, pp. 1016-1064.
- HARRISON, C. C., and RAMSAY, G. C. (1933). *J. Trop. Med. Hyg.*, **36**, 3, pp. 33-41.



- IYENGAR, M. O. T. (1930) .. *Ind. J. Med. Res.*, **17**, pp. 1171-1188.  
 DE JESUS, P. I. (1936) .. *Philippine Jour. Sci.*, **59**, 4, pp. 473-491.  
     *Idem* (1937) .. *Ibid.*, **62**, 2, pp. 125-136.  
 KAPESZKY, E. (1940) .. *Arch. Schiffs. u. Tropen. Hyg.*, **44**, pp. 103-119.  
 MANSON, D. (1936) .. *Ind. Med. Gaz.*, **71**, 5, pp. 270-275.  
     *Idem* (1939) .. *J. Mal. Inst. Ind.*, **2**, 1, pp. 85-95.  
 PETERSON, W. H., FRIED, E. B., and DOMOGALLA, B. P. (1925). *Jour. Biol. Chem.*, **63**, pp. 287-295.  
 PUNTONI, V. (1934) .. *R. C. Accad. naz. Lincei, Cl. Sci. fis. mat. nat.* (6), **19**, 6, pp. 428-432. Rome. Also *Rev. App. Ent.*, **B**, **32**, p. 133.  
 RAMSAY, G. C. (1930) .. *J. Trop. Med. Hyg.*, **33**, pp. 352-357.  
 RAMSAY, G. C., and MACDONALD, G. (1936). *Ind. Med. Gaz.*, **71**, pp. 699-710.  
 RUSSELL, P. F., and JACOB, V. P. (1939). *J. Mal. Inst. Ind.*, **2**, 3, pp. 293-313.  
 RUSSELL, P. F., and MOHAN, B. N. (1940). *Amer. J. Hyg.*, **31**, 1, pp. 19-25.  
 SENIOR WHITE, R. (1926) .. *Bull. Ent. Res.*, **16**, pp. 187-248.  
     *Idem* (1928) .. *Ind. J. Med. Res.*, **16**, 1, pp. 11-30.  
     *Idem* (1936) .. *Rec. Mal. Surv. Ind.*, **6**, 3, pp. 439-445.  
 SKOPINTZEV, B. A., and OVCHINNIKOVA, Y. S. (1933). *J. Appl. Chem. (U. S. S. R.)*, **6**, pp. 1173-1179. Also *Chem. Abst.*, **28**, p. 4143.  
 STAS, M. E. (1925) .. *Chem. Weckblad.*, **22**, pp. 584-585. Also *Chem. Abst.*, **20**, p. 290.  
 THOMSON, R. C. M. (1940a) .. *J. Mal. Inst. Ind.*, **3**, pp. 265-294.  
     *Idem* (1940b) .. *Ibid.*, pp. 295-322.  
     *Idem* (1940c) .. *Ibid.*, pp. 323-348.  
 WILLIAMSON, K. B. (1927) .. *Trans. Far East. Ass. Trop. Med.*, 7th Cong., **2**, pp. 723-735.  
     *Idem* (1928) .. *Bull. Ent. Res.*, **18**, pp. 433-459.  
     *Idem* (1935) .. The control of rural malaria by natural methods. 26 pp. L. of N. Eastern Bureau, Singapore.  
     *Idem* (1936) .. References and notes supplementary to the 'The control of rural malaria by natural methods'. Series I-III. 89 pp. with 3 plates. L. of N. Eastern Bureau, Singapore.



## OBSERVATIONS ON *ANOPHELES LEUCOSPHYRUS* IN THE DIGBOI AREA, UPPER ASSAM.

BY

R. H. P. CLARK, M.D., M.R.C.P., D.T.M. & H.  
(*Medical Officer, Assam Oil Company, Ltd.*),

AND

M. A. CHOUDHURY, L.M.P.  
(*Health Officer, Assam Oil Company, Ltd.*).

[March 11, 1941.]

### INTRODUCTION.

SINCE 1937, the Digboi area has been under continuous malarial survey by the personnel of the Assam Oil Company's Medical staff, except for six months in 1939. In July 1938, Dr. W. B. Crawford first noted a *Plasmodium* infection in *A. leucosphyrus*, a finding since repeated on many occasions. These and other observations are now presented, as they appear to incriminate this anopheline as a not unimportant local vector.

### TOPOGRAPHY AND METEOROLOGY.

Digboi is situated in Lakhimpur District, Assam, 27° 23" N., 95° 37" E. The main area to be considered is about 10 square miles in extent, and occupies a wide bay encroaching into dense hilly jungle. The bay is open to extensive flat ill-drained paddy land. The contained area, lying at a slightly higher level, is flat, well-drained, grassed and largely built over. It is separated from the forest by a bordering zone, which is hilly, cut up by deep nullahs, and partially or completely cleared of jungle; in this area, many bungalows and quarters are situated.

The Kharjan area is a mile-long section of a road which traverses dense forest about 3 miles from Digboi. The road was opened in late 1938, and since that time about 40 families of wood-cutters have settled by the roadside, finding employment in the forest, which they are slowly clearing. This area came under our notice only from May 1940. In June, all the local children, 15 in number, were examined and all showed spleen enlargement up to 7 fingers. The adults were not examined; they stated that 'fever' was extremely common.



## METEOROLOGICAL RECORDS.

Meteorological records, taken at Digboi for 1938 and 1940, are shown in Table I, together with the percentage of malarial incidence among Assam Oil Company employees working in Digboi.

TABLE I.

Month.	1938.				1940.				PERCENTAGE INCIDENCE OF MALARIA AMONGST A. O. C. EMPLOYEES.	
	Average temperature, maximum.	Average temperature, minimum.	Average relative humidity, per cent.	Rainfall in inches.	Average temperature, maximum.	Average temperature, minimum.	Average relative humidity, per cent.	Rainfall in inches.	5,670 in 1938.	5,570 in 1940.
January ..	70	53	..	3.42	72	47	89	0.61	0.14	0.52
February ..	74	54	..	5.21	75	53	87	4.63	0.12	0.35
March ..	76	61	..	13.05	72	59	88	10.48	0.05	0.35
April ..	87	67	..	8.16	88	65	81	6.22	0.15	0.26
May ..	91	72	..	8.01	87	71	86	18.84	0.43	0.48
June ..	91	74	..	33.95	91	73	85	20.55	0.46	0.80
July ..	90	75	..	28.50	91	77	85	21.85	0.95	1.00
August ..	92	77	83	16.70	95	77	86	10.32	1.45	1.41
September ..	80	76	83	23.31	89	74	89	13.27	1.09	1.65
October ..	87	71	83	6.45	88	70	86	5.90	1.21	2.10
November ..	76	58	83	2.25	78	60	87	..	1.16	1.83
December ..	73	52	88	0.02	73	54	88	0.58	0.33	0.70

DISSECTION RECORDS OF *A. LEUCOSPHYRUS*.

The results of 859 dissections of *A. leucosphyrus* in 1938 and 1940 are given in Table II, together with figures for *A. minimus* for this period.

HABITS OF ADULT *A. LEUCOSPHYRUS*.

Adult catches were made in the mornings. In every case, the insect was caught in a human habitation, and in several cases in the inside of mosquito nets. No males were found. In the Digboi area, *A. leucosphyrus* was most common in the hilly zone bordering the forest, which has been under *A. minimus* control for some years and in which this species is now seldom found. In 1940, cow-sheds in an area, where *A. leucosphyrus* was common, were searched twice



TABLE II.

Month.	DIGBOI AREA.										KHARJAN AREA.					
	1938.					1940.					1940.					
	Total catch.	Number dissected.	Infected in gut only.	Infected in gland only.	Infected in both gut and gland.	Total catch.	Number dissected.	Infected in gut only.	Infected in gland only.	Infected in both gut and gland.	Total catch.	Number dissected.	Infected in gut only.	Infected in gland only.	Infected in both gut and gland.	
<i>A. leucosphyrys</i> record.																
January	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	
February	..	..	..	..	..	3	3	..	..	..	..	..	..	..	..	
March	..	..	..	..	..	1	1	..	..	..	..	..	..	..	..	
April	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	
May	..	..	..	..	..	5	3	..	..	..	28	13	..	..	..	
June	..	..	..	..	..	26	25	..	2	..	331	293	4	10	1	
July	..	85	55	1	1	1	37	29	..	2	..	157	122	..	1	..
August	..	98	36	1	..	..	13	11	..	..	..	196	114	..	1	..
September	..	25	23	..	..	..	8	7	..	..	..	93	75	1	1	..
October	..	24	20	..	1	..	4	3	..	..	..	28	24	1	..	..
November	..	1	1	..	..	..	..	..	..	..	..	..	..	..	..	..
December	..	..	..	..	..	..	..	..	..	..	1	1	..	..	..	..
TOTAL	..	233	135	5 (3.7%)		97	82	4 (4.9%)			834	642	20 (3.1%)			
<i>A. minimus</i> record.																
TOTAL	..	298	277	9 (3.2%)		102	98	3 (3.0%)			36	33	1 (3.0%)			

a week from June to August without results. It may be noted that, in the Kharjan area, no cattle or goats were kept, the only domestic animals being dogs and fowls.

#### BREEDING HABITS.

The larvæ of *A. leucosphyrys* were found in small collections of stagnant water in open jungle. These were small pools in marshy areas, and footprints



of elephants (Plate XIX, figs. 1 and 2; Plate XX, fig. 3). Dense jungle has not been thoroughly investigated. In no case were larvæ found in completely unshaded areas or in running water, either shaded or unshaded. Neither were they found in partially shaded or open tanks, where *A. vagus*, *A. philippinensis* and *A. kochi* are common breeders.

TABLE III.  
*Records of catches of A. leucosphyrus larvæ.*

Month.	DIGBOI AREA.		KHARJAN AREA.		Month.	DIGBOI AREA.		KHARJAN AREA.	
	Number of larvæ found.		Number of larvæ found.			Number of larvæ found.		Number of larvæ found.	
	1938.	1940.	1938.	1940.		1938.	1940.	1938.	1940.
January ..	..	..	..	..	July ..	25	64	..	10
February ..	13	..	..	..	August ..	..	121	..	14
March ..	4	9	..	..	September ..	21	23	..	29
April ..	12	2	..	..	October ..	..	23	..	..
May ..	8	..	..	15	November ..	..	74	..	18
June ..	33	181	..	13	December ..	..	..	..	..
TOTAL ..					116	497	..	99	

#### IDENTIFICATION.

Identification of adult specimens of *A. leucosphyrus* was confirmed through the kindness of the Director, Pasteur Institute, Shillong. Larvæ were bred out to confirm the species.

Sporozoites were submitted to the Assam Medical Research Society, and by them forwarded to the Malaria Institute of India, who were of the opinion that they were of the human type.

#### SUMMARY AND CONCLUSION.

The results of a malaria survey in and near Digboi during 1938 and 1940 are given.

In particular *A. leucosphyrus* yielded a Plasmodium infection rate of 3.1 to 4.9 per cent in areas where *A. minimus* is the less plentiful species. In one particular area (Kharjan), the small local population had a very high incidence of malaria.

The habits and breeding places of *A. leucosphyrus* are commented on.

It is concluded that *A. leucosphyrus* is a vector species of importance in Digboi and its vicinity.



PLATE XIX.



Fig. 1. Pool in marshy area forming a breeding place for *A. leucosphyrus*.



Fig. 2. Small pool in partially shaded jungle, a breeding place for *A. leucosphyrus*



PLATE XX.



Fig. 3. Swampy area with footprints of elephants forming breeding places for *A. leucosphyrus*.



ACKNOWLEDGMENTS.

The authors thank Dr. D. K. Viswanathan of the Assam Medical Research Society, and the Director, Malaria Institute of India, for help in identification; and the General Manager of the Assam Oil Company for permission to publish these findings.







# THE INSECTICIDAL AND LARVICIDAL ACTION OF THE ESSENTIAL OILS OF *OCIMUM BASILICUM* (LINN.) AND *OCIMUM SANCTUM* (LINN.).

BY

R. N. CHOPRA,

D. N. ROY,

AND

S. M. GHOSH.

(School of Tropical Medicine, Calcutta.)

[March 11, 1941.]

IN continuation of our work on the insecticidal value of indigenous plants reputed to possess such properties, we now present a report on the action of *Ocimum*, which not only grows wild throughout India, Burma and Ceylon but is also cultivated in many places. Almost all parts of this plant, including its seeds, are extensively used in this country for the treatment of various diseases, especially bronchitis. The dried leaves are often burnt on cow-dung for the purpose of repelling mosquitoes. Such beneficial properties are generally ascribed mainly to the essential oils, the proportion of which varies widely in different species of this plant.

We had opportunities of testing *Ocimum basilicum* Linn., and two varieties of *O. sanctum* Linn., commonly known as *Sri tulsi* and *Krishna tulsi*. The chemical composition of the oils is shown in Table I.

TABLE I.

Plant.	Percentage yield of essential oil.	Percentage phenol.	Percentage aldehyde.
<i>Ocimum basilicum</i> .. ..	0.20 to 0.48	7.4 to 19.0	5 to 35
<i>Ocimum sanctum</i> ( <i>Sri tulsi</i> ) ..	0.20 to 0.33	50.0 to 75.8	10 to 15
<i>Ocimum sanctum</i> ( <i>Krishna tulsi</i> ) ..	0.10 to 0.23	45.0 to 76.1	15 to 25



The strength of the essential oil in the concentrated form used by us for tests on mosquitoes and flies was 10 per cent; this was diluted with varying proportions of kerosene. The technique followed has already been outlined in a previous communication (Chopra, Roy and Ghosh, 1940).

*Ocimum basilicum* Linn.

The essential oil is yellowish green and highly volatile and if kept unsealed, it solidifies. In addition to the constituents described before, it contains according to Van Romburgh and Enklaar (1904), a new terpene, ocimene ( $C_{10}H_{16}$ ), closely resembling myrecene.

The seeds yield to petroleum ether 9.5 per cent of oil which has a specific gravity at 25°C., 0.9333 and its refractive index 1.48, iodine, saponin and acetyl value, 166.6, 191.8 and 16.5 respectively (Ramaswamy Ayyar and Patwardhan, 1930). We have not used the oil extracted from the seeds, and in all our observations we obtained our supply from the leaves alone. Its action on different insects is shown in Table II.

TABLE II.  
The action of *O. basilicum* on different insects.

Strength of the oil used.	PERCENTAGE KILL OF DIFFERENT INSECTS.				
	<i>Aedes aegypti</i> .	<i>Culex fatigans</i> .	House flies.	Blue-bottle flies.	Cockroaches.
Ten per cent essential oil: full concentration.	40 to 45	60 to 64	5 to 8	10 to 15	Nil
Ten per cent oil diluted with equal parts of kerosene 1 : 2.	35 to 36.5	48 to 50	3 to 4	8 to 12	Nil
Ten per cent oil diluted with 4 parts of kerosene 1 : 5.	10 to 12	20 to 23	Nil	Nil	Nil

*Ocimum sanctum* Linn. (*Sri tulsi* and *Krishna tulsi*.)

These two plants, though they scarcely deserve to be considered as varieties, are easily distinguished by the colour of their leaves, one being whitish (Bengali name : *Sri tulsi*) and the other black (Bengali name : *Krishna tulsi*). The English name, *Holy basil*, is common to both. The essential oil distilled from the leaves of both these plants, though has the same chemical constituents, yet differs greatly in the proportion of phenol and aldehyde contents; *Sri tulsi* having high phenol and *Krishna tulsi* having high aldehyde contents.

They are distributed not only in India, Burma and Ceylon, but also in the Malay Archipelago, Australia, Pacific Islands, and in Western Asia and Arabia. In India, they are found in the Himalayas up to an altitude of 6,000 feet. Although common in waste lands, they are more frequently cultivated, and it is thus doubtful whether this species is really indigenous to India (Watt, 1891).

The essential oils of both *Sri* and *Krishna tulsi* contain methyl chavicol, cineole and linalol (Finnemore, 1926).



The action of the essential oil obtained from the leaves of these two plants is shown in Table III.

TABLE III.  
Insecticidal action of the two varieties of *O. sanctum*.

Strength of the oil used.	PERCENTAGE KILL OF DIFFERENT INSECTS.				
	<i>Culex fatigans</i> .	<i>Aedes ægypti</i> .	House flies.	Blue-bottle flies.	Cockroaches.
Concentrated, i.e., 10 per cent :—					
<i>Sri tulsi</i> ..	65 to 68	44 to 46	12 to 15	20 to 22	Nil
<i>Krishna tulsi</i> ..	60 to 65	42 to 43	10 to 11.5	18 to 20	Nil
Diluted with kerosene, 1 : 2 :—					
<i>Sri tulsi</i> ..	55 to 56	38 to 40	6 to 9	15 to 16.5	Nil
<i>Krishna tulsi</i> ..	40 to 45	28 to 30	3 to 4	12 to 14.5	Nil
Diluted with kerosene, 1 : 5 :—					
<i>Sri tulsi</i> ..	15 to 20	9 to 10	Nil	2 to 5	Nil
<i>Krishna tulsi</i> ..	8 to 9	4 to 5	Nil	Nil	Nil

#### MOSQUITO REPELLENT ACTION.

The strong odour of the oil is disagreeable to *Culex fatigans* and *Armigeres obturbans*, but not to *Aedes ægypti*. When rubbed on the hand, it will ensure comparative freedom from their bite for about half an hour. In the case of *Sri tulsi*, the action lasts a little while longer.

#### LARVICIDAL ACTION.

Both *O. basilicum* and *O. sanctum* possess larvicidal action to a marked degree. When used in laboratory experiments in the concentrated form, it acts on larvæ of *Culex*, *Anopheles* and *Aedes*, which are all killed in the course of two hours. The larvicidal properties of *Sri tulsi* are definitely stronger than those of the others.

#### FUMIGATION.

Fumigation with dried leaves does not seem to possess any marked mosquito repellent action, neither are mosquitoes killed thereby.

#### CONCLUSIONS.

The foregoing results clearly indicate that both *O. basilicum* and *O. sanctum* possess some insecticidal action, which is marked in the case of mosquitoes, though it cannot be compared with that of pyrethrum. The mosquito repellent



action of the oil lasts for about two hours. The fume from burning dried leaves on cow-dung has no more action than when cow-dung is burnt alone. From the practical point of view, their larvicidal action when used alone does not merit much consideration owing to the high cost of manufacture.

## REFERENCES.

- CHOPRA, R. N., ROY, D. N., and J. *Mal. Inst. Ind.*, **3**, **4**, pp. 495-498.  
GHOSH, S. M. (1940).  
FINNEMORE, H. (1926) .. .. The essential oils, pp. 738-741. Ernest Benn, Ltd., London.  
RAMASWAMY AYYAR, P., and PATWARDHAN, V. A. (1930). *Proc. 17th Ind. Sci. Cong.*, p. 185.  
VAN ROMBURGH, P., and ENKLAAR, C. J. (1904). *J. Soc. Chem. Industry*, **23**, p. 1235.  
WATT, G. (1891) .. .. Dictionary of the economic products of India, **5**, pp. 440 and 443. W. H. Allen and Co., London.



# AQUATIC PLANTS IN THE ECOLOGY OF ANOPHELINE MOSQUITOES.

BY

P. SEN, M.Sc. (Cal.), Ph.D. (Lond.), D.I.C.  
(Entomologist, Bengal Public Health Department, Calcutta).

[March 17, 1941.]

## CONTENTS.

	PAGE
I INTRODUCTION .. .. .	113
II METHODS .. .. .	114
III FIELD OBSERVATIONS .. .. .	114
A. Different plants studied and their association with anopheline mosquitoes	117
1. <i>Spirogyra</i> sp. .. .. .	117
2. <i>Ceratophyllum demersum</i> L. .. .. .	117
3. <i>Utricularia flexuosa</i> Vahl. .. .. .	118
4. <i>Hydrilla verticillata</i> Casp. .. .. .	119
5. <i>Pistia stratiotes</i> L. .. .. .	120
6. <i>Ipomoea reptans</i> Poir. .. .. .	120
7. <i>Lemna minor</i> L. .. .. .	121
8. <i>Limnanthemum cristatum</i> Griseb. .. .. .	122
9. <i>Najas foveolata</i> A. Br. .. .. .	122
10. <i>Ottelia alismoides</i> Pers. .. .. .	122
11. <i>Hymenachne myurus</i> Beauv. .. .. .	123
12. <i>Eichornia speciosa</i> Kunth. .. .. .	123
B. Preferential plant association with different anopheline mosquitoes	125
C. Anopheline breeding and mosquito output in association with aquatic plants	132
D. Changes in the anopheline fauna accompanying the changes of flora in a tank	132
IV. DISCUSSION .. .. .	133
V. SUMMARY .. .. .	135
ACKNOWLEDGMENTS .. .. .	136
REFERENCES .. .. .	136

## I. INTRODUCTION.

VERY little work has been done to establish the relationship of the breeding of the different species of anopheline mosquitoes with the various aquatic vegetation usually present in tanks.



It has often been observed that catches of adult anophelines in a locality are very low as compared with their intensive breeding in the nearby tanks. Ecological conditions may not be suitable for the pupation of the larvæ or the emergence of the adults (Sen, 1935). The present paper deals not only with breeding preferences, but also with the percentage of emergences of different species of anophelines in association with the various forms of aquatic vegetation met with in an ordinary tank in Bengal.

In conducting such an investigation, two conditions must be fulfilled: (1) the study must be carried out in an area where the mosquito breeding places are not subjected to human intervention; and (2) the breeding places selected must contain the vegetation to be studied in more or less pure culture. The second condition is not always easy to fulfil, since the flora of a tank in the tropics are seldom unmixed and vary considerably with the changes of the seasons. The observations, therefore, in the case of any particular aquatic plant, had to be limited to the period during which this was not associated with other forms of vegetation. The entire series of observations, however, was continued throughout a period of 12 months, from February 1936 to January 1937.

## II. METHODS.

Tanks, or portions thereof, containing a particular plant community unassociated with others, as far as possible, were selected for investigation. Ordinary mosquito nets, measuring 6 feet 9 inches  $\times$  3 feet 4 inches, were fixed over such selected areas towards the evening (Plate XXI). The nets were removed on the following morning, and any anophelines which had emerged within them during the night were identified and recorded. The nets were tied either to the branches of overhanging trees or to poles driven into the bed of the tanks, weights being fixed to the corners of each net to prevent it from being carried away by the wind. From 6 to 12 inches of the lower edge of the net remained under water, so that there was little chance of larvæ or pupæ entering from outside. The species of anophelines breeding in the areas was ascertained by means of larval collections made immediately before the nets were placed in position, the records being based on six dips with an aluminium fry-pan 6 inches in diameter.

## III. FIELD OBSERVATIONS.

Observations were carried out in four villages, Chakraghata, Doharia, Guchuria and Chandnagar, situated on both sides of the railway station Madhyamgram on the Khulna section of the Eastern Bengal Railway, within ten miles of Calcutta. Detailed descriptions of the breeding places under observation are given in Table I. The villages were highly malarious, the spleen rate of the children being above 50 per cent in the malaria season, and were not subjected to any form of antimalarial operations.

The different species of aquatic vegetation studied around each village and the number of observations carried out in each case are recorded in Table II. It will be noted that, in association with twelve kinds of aquatic plants, only twelve species of anophelines were encountered. Table II also gives the total number of emergences of anophelines and the number of breeding places observed in relation to each type of vegetation.



PLATE XXI.



Mosquito curtain trap in position in a tank (T<sub>2</sub>) at  
Gachuma with *Ipomoea repens*







TABLE I.  
Description of breeding places examined at Madhyamgram.

Village.	Nature and reference number of breeding places.	Dimensions (ft. × ft.).	Average depth of water (ft.).	Whether with shade or not.	Vegetations with period of observation.
Guchuria ..	Tank (1)	90 × 80	12	Sunny	<i>Hydrilla</i> (May to July).
	Tank (2)	45 × 40	4	"	<i>Ipomæa</i> (May and June).
	Tank (3)	250 × 100	10	Partly shaded.	<i>Utricularia</i> (May and July); <i>Limnanthemum</i> (June); <i>Ipomæa</i> (July).
Chandnagar ..	Tank (1)	200 × 50	3	"	<i>Limnanthemum</i> (February to April); <i>Hymenachne</i> (July)
Chakraghata	Tank (2)	315 × 160	15	"	<i>Spirogyra</i> (February and March); <i>Ceratophyllum</i> (April to June); <i>Hymenachne</i> (July).
	Tank (7)	250 × 100	4	"	<i>Limnanthemum</i> (April and May); <i>Lemna</i> (September).
	Tank (11)	60 × 35	4½	Sunny	<i>Pistia</i> (September).
	Tank (11A)	95 × 60	7	"	<i>Pistia</i> (October and November).
	Tank (18)	150 × 75	6	Partly shaded.	<i>Pistia</i> (December).
	Tank (14A)	320 × 200	4½	"	<i>Hydrilla</i> (January).
Doharia ..	Tank (6)	60 × 50	2½	Sunny	<i>Ipomæa</i> (February and March); <i>Spirogyra</i> (April).
	Tank (21)	150 × 90	5	"	<i>Pistia</i> (February to July, September and October); <i>Utricularia</i> (May).
	Tank (18)	125 × 70	6	Partly shaded.	<i>Limnanthemum</i> (March).
	Tank (25)	30 × 30	3	"	<i>Eichornia</i> (April and May).
	Tank (26)	250 × 90	3½	"	<i>Ceratophyllum</i> (April and May); <i>Lemna</i> (May); <i>Hymenachne</i> (June).
	Tank (23)	75 × 45	6	Sunny	<i>Lemna</i> (May).
	Tank (17)	300 × 60	7½	Partly shaded.	<i>Pistia</i> (June).
	Tank (28)	225 × 125	9	Sunny	<i>Pistia</i> (July to September and November).
	Pond (2A)	60 × 30	4	Shaded	<i>Pistia</i> (July to September and November).
	Tank (16)	155 × 100	3	Partly shaded.	<i>Hymenachne</i> (August).
	Tank (15)	75 × 65	4½	Sunny	<i>Pistia</i> (August and September).
	Tank (27A)	120 × 75	5	"	<i>Pistia</i> (November and December).
	Pond (A)	40 × 15	2	"	<i>Utricularia</i> (November to January).
	Tank (4)	60 × 45	4½	Shaded	<i>Ipomæa</i> (November and December).
	Tank (10)	60 × 21	6½	Sunny	<i>Najas</i> (December and January).
	Tank (9)	75 × 45	3½	"	<i>Pistia</i> (December); <i>Ottelia</i> (December and January).
	Tank (20)	90 × 30	3	"	<i>Utricularia</i> (January).
	Tank (27B)	60 × 60	6½	Shaded	<i>Pistia</i> (January).

Note.—Shaded denotes overhanging trees on all sides of the tanks. Partly shaded denotes trees on less than four sides.



TABLE II.  
Vegetation and the number of observations carried out in each village at Madhyamgram.

Name of vegetation.	GUCHURIA.			CHANDNAGAR.			CHAKRACHATA.			DOHARIA.			ENTIRE AREA.		
	Number of breeding places examined.	Number of observations carried out.	Number of times of Anopheles emergence under traps.	Number of breeding places examined.	Number of observations carried out.	Number of times of Anopheles emergence under traps.	Number of breeding places examined.	Number of observations carried out.	Number of times of Anopheles emergence under traps.	Number of breeding places examined.	Number of observations carried out.	Number of times of Anopheles emergence under traps.	Number of breeding places examined.	Number of observations carried out.	Number of times of Anopheles emergence under traps.
<i>Hydrilla verticillata</i> Casp.	1	27	22	..	..	..	1	3	2	..	..	..	2	30	24
<i>Ipomoea reptans</i> Poir.	2	15	7	..	..	..	..	..	..	2	16	5	4	31	12
<i>Limnanthemum cristatum</i> Griseb.	1	14	13	1	10	7	1	12	8	1	7	1	4	43	29
<i>Utricularia flexuosa</i> Vahl.	1	7	6	..	..	..	..	..	..	3	40	20	4	47	26
<i>Hymenachne myosuus</i> Beauv.	..	..	..	1	3	nil	1	5	2	2	5	nil	4	13	2
<i>Ceratophyllum demersum</i> L.	..	..	..	..	..	..	1	48	45	1	5	4	2	53	49
<i>Pistia stratiotes</i> L.	..	..	..	..	..	..	3	32	18	8	181	143	11	213	161
<i>Lemna minor</i> L.	..	..	..	..	..	..	1	2	nil	2	9	6	3	11	6
<i>Spirogyra</i> sp.	..	..	..	..	..	..	1	29	28	1	13	12	2	42	40
<i>Eichornia speciosa</i> Kunth.*	..	..	..	..	..	..	..	..	..	1	6	2	1	6	2
<i>Ottelia alismoides</i> Pers.	..	..	..	..	..	..	..	..	..	1	16	5	1	16	5
<i>Najas foveolata</i> A. Br.	..	..	..	..	..	..	..	..	..	1	25	9	1	25	9
TOTAL	5	63	48	2	13	7	9	131	103	23	323	207	39	530	365

\* This plant was also studied at Sonarpur in four breeding places, and 234 observations were carried out.  
Note.—The number of times breeding of anophelines recorded corresponds with the number of observations carried out.



## A. DIFFERENT PLANTS STUDIED AND THEIR ASSOCIATION WITH ANOPHELINE MOSQUITOES.

### 1. *Spirogyra* sp.

*Spirogyra* is a green alga found floating in masses on the surface of water, with which are usually associated several scattered unicellular and colonial algæ. The anopheline association with this alga was studied during 1936, from February to April. After this, other forms of vegetation, especially *Ceratophyllum demersum* Linn., made their appearance. Forty-two observations were carried out in two tanks, one in Doharia (Tank 6)\* and the other in Chakraghata (Tank 2). Towards the end of March, *Ceratophyllum* appeared in the tank at Chakraghata, and the tank at Doharia, where *Spirogyra* appeared in April after it had been partially cleared of *Ipomœa*, its former plant inhabitant, almost dried up with the approach of May.

Larvæ of nine species of anophelines, *A. hyrcanus* var. *nigerrimus* Giles, *A. barbirostris* Van der Wulp, *A. ramsayi* Covell, *A. annularis* Van der Wulp, *A. philippinensis* Ludlow, *A. pallidus* Theobald, *A. subpictus* Grassi, *A. vagus* Dönitz and *A. aconitus* Dönitz, were recorded in the tanks during the period under observation, whereas seven species only, *A. subpictus*, *A. annularis*, *A. hyrcanus* var. *nigerrimus*, *A. philippinensis*, *A. ramsayi*, *A. vagus* and *A. culicifacies* Giles, were represented in the catches made in the mosquito net-trap, in that order of numerical abundance.

Although no specimens of *A. culicifacies* were recorded as larvæ, a single adult of this species was encountered out of 210 of all species taken in the trap in 3 months. On the other hand, although larvæ of *A. barbirostris*, *A. pallidus* and *A. aconitus* were captured in scanty numbers, none of these appeared in the trap catches as adult insects. The single specimen of *A. culicifacies* captured emerged in April, when the weather had definitely warmed up.

### 2. *Ceratophyllum demersum* L.

These are perennial submerged plants, having fragile branches with whorls of filiform leaves, and are of greenish or pale brown colour. The study of anopheline association with this plant was taken up during the period April to June. After this, owing to heavy rains, the plants became less visible and further observations could not be continued. Forty-three observations were carried out in two tanks, one in Doharia (Tank 6) and the other in Chakraghata (Tank 2). The tank at Chakraghata was under observation for *Spirogyra* up to the end of March, when *Ceratophyllum* appeared. From April onwards this tank was observed for *Ceratophyllum* until June, when it was cleaned for pisciculture purposes. The tank at Doharia, on the other hand, became covered with *Lemna* towards the latter half of May, and no further observations on *Ceratophyllum* could be carried out.

Seven species, *A. annularis*, *A. philippinensis*, *A. pallidus*, *A. jamesi*, *A. subpictus*, *A. vagus* and *A. culicifacies*, were recorded as larvæ in association with *Ceratophyllum*. Specimens of all these, except *A. pallidus* and *A. culicifacies*, the larvæ of which were present in very scanty numbers, emerged as adults from among the vegetation. A single adult *A. culicifacies*,

---

\*The number against a breeding place represents the serial number entered in the survey maps, not reproduced (cf. Table I).



however, was obtained in a trap from the same tank in April, when the observations on *Spirogyra* were in progress, and it is possible that the present record of *A. culicifacies* larvæ in association with *Ceratophyllum* was due to undetected contamination of the latter plant with this alga, which experience has shown affords a very favourable breeding medium of the species, at least in the laboratory. Two more species, *A. hyrcanus*\* and *A. ramsayi*, were also recovered as adults from the traps over this type of vegetation, although their larvæ were not recorded before the traps were set in position. The emergence of *A. annularis* was very abundant during the period the observations were carried out. Out of a total trap collection of 316 Anopheles of all species, as many as 287 were of this species (nearly 91 per cent).

The maximum breeding, as also the maximum emergence, occurred during May. In this month, 352 larvæ out of 608 for the entire period, and 194 emergences out of 316, were recorded. In June, only *A. annularis* was found breeding in the area, while the emergence of this species was out of proportion to the larval density, 47 adults being captured as against 19 larvæ as the result of twelve observations. This was possibly due to a preponderance of pupæ, which were not recorded in the larval dips.

### 3. *Utricularia flexuosa* Vahl.

This plant belongs to the group commonly known as the bladder-worts, which have a reputation for capturing and digesting as food small insects and their larvæ in the bladders borne at the base of their leaves. *Utricularia flexuosa* is a free floating plant with multifid leaves, but, as with *Ceratophyllum*, accidental attachment or rather anchorage frequently occurs through entanglement with other plants, or by being partly stuck in the mud when the water is shallow.

The study of the association of this plant with anopheline breeding and emergence was taken up during May, when it was first seen in a tank at Doharia (Tank 21). This tank was under observation for *Pistia* until April, when this was removed. In June, *Pistia* again became predominant and the observations on *Utricularia* had to be stopped. The observations were, however, resumed in July in a tank at Guchuria (Tank 3). After this, from August to October, the traps could not be used, either owing to heavy rains or because the plants remained below the water surface. From November to January, the observations were continued in two more breeding places in Doharia (Tank 20 and Pond A). Forty-seven observations were carried out in the four breeding places cited.

Eight species of anophelines, *A. hyrcanus*, *A. annularis*, *A. philippinensis*, *A. pallidus*, *A. ramsayi*, *A. jamesi*, *A. subpictus* and *A. vagus*, were found breeding in association with *Utricularia*, of which only the first four species emerged. Larvæ of the other four species were, however, taken in scanty numbers only. One additional species, *A. barbirostris*, was obtained as adults (four specimens only) in January 1937 from traps placed over *Utricularia*, indicating that this species also is capable of breeding in association with the plant.

The most numerous larval catch was that of *A. annularis* (296), followed by *A. pallidus* (248), *A. philippinensis* (99) and *A. hyrcanus* (56). As regards

---

\* All references to *A. hyrcanus* in this paper refer to *A. hyrcanus* var. *nigerrimus*.



emergences, *A. annularis* (40) again topped the list, followed by *A. philippinensis* (8) and *A. hyrcanus* (7). In the entire period under observation, only one emergence of *A. pallidus* (December 1936) was recorded, although the larval density of the species was by no means insignificant. The rate of anopheline emergence in general in association with this plant was very poor, since in forty-seven observations covering 5 months, including three distinct seasonal changes, i.e., summer in May, rains in July and winter conditions from November to January, only 60 adults emerged, as compared with 713 larvæ captured during the same period. Another striking feature was that, while the anopheline breeding increased from May to December, the emergence rate decreased. In twelve observations during May, 81 larvæ were collected and 24 emergences recorded, whereas in December, as the result of eleven observations, as many as 339 larvæ were collected and only 19 emergences recorded. *Utricularia*, therefore, does not prevent the breeding of different species of anophelines, especially *A. annularis* and its allied species, but the presence of the plants is associated with a considerable reduction in the number of emergences of the various species.

#### 4. *Hydrilla verticillata* Casp.

This is a submerged aquatic herb with slender stems, having many branches and leaves arranged in whorls. The plant is attached to the soil and is usually found associated with several species of floating algæ. Towards the close of spring, owing to the paucity of algæ, opportunity was afforded to study the association of various anophelines with an almost pure community of *Hydrilla*. Thirty observations were carried out in two tanks, one at Guchuria (Tank 1) and the other at Chakraghata (Tank 14A), during the period May to July 1936, and again in January 1937. After July, observations could not be continued owing to heavy rains, when the plants remained more or less submerged.

The various species found to breed in association with this plant were *A. hyrcanus*, *A. annularis*, *A. philippinensis*, *A. pallidus*, *A. subpictus* and *A. vagus*. All these species, with the exception of *A. hyrcanus* and *A. pallidus*, were taken as adults in trap collections. *A. ramsayi*, which was not recorded in its larval stage, was represented as adults in trap collections during every month from May to July. Probably some pupæ entered under the trap-net undetected from some other part of the tank where *Pistia*, a favourite plant for the breeding of this species, flourished.

The maximum breeding as also the maximum emergence in association with *Hydrilla* were noticed during May and June. In May, 142 anopheline larvæ and 56 emergences, as the result of six observations, were recorded, while in June, 241 larvæ and 44 emergences were encountered in fifteen observations. In July, the breeding was very much retarded, only 67 larvæ being obtained in six observations and with a consequent fall in the emergence rate, only 7 adult anophelines being captured during that month; while in January, the emergences were still less, only two *A. annularis* mosquitoes having emerged as the result of three observations against a record of 39 larvæ, of which 32 were *A. annularis*. Judged from larval density *A. annularis* and *A. philippinensis* were almost the only mosquitoes recorded as breeding in association with this plant, larvæ of the former species being by far the more abundant. Out of 489 larvæ captured, 439 were *A. annularis* and 30 *A. philippinensis*. The emergences recorded for these two species were 93 for *A. annularis* and 3 for *A. philippinensis*, out of a



total of 109 recorded as the result of thirty observations during the 4 months May to July 1936 and January 1937.

#### 5. *Pistia stratiotes* L.

This plant, commonly known as the water-lettuce, was present in abundance throughout the year. It floats freely on the water surface, being moved about by the wind, and multiplies prolifically. The number of observations carried out was consequently high, 213 being distributed over 11 tanks, of which 8 were in Doharia (Tanks 9, 15, 17, 21, 27A, 27B, 28 and Pond 2A) and 3 in Chakra-ghata (Tanks 11, 11A and 18).

Eleven species of anophelines, *A. hyrcanus*, *A. barbirostris*, *A. ramsayi*, *A. annularis*, *A. philippinensis*, *A. pallidus*, *A. subpictus*, *A. vagus*, *A. varuna* Iyengar, *A. aconitus* and *A. jamesi*, were found as larvæ in association with this plant, all of which with the exception of *A. pallidus* emerged.

The maximum breeding as also the maximum number of emergences occurred during May and June, when the average larval incidence per observation was 18 and 26 respectively, whereas the average for emergences was 12 and 8 respectively. In July, the average larval incidence and adult emergences per observation were 16 and 5 respectively. After this, the breeding of anophelines in the *Pistia* tanks fell considerably, showing an average of 7 larvæ per observation from August to October with short rises in November and December, when 12 and 15 larvæ respectively per observation were recorded. The emergence rate fell appreciably after July, the catches producing only 3 mosquitoes per observation during August to September, 2 in October and 1 each in November and December. In January, with the fall of temperature, emergences came to an end altogether as calculated from the records of seven observations during the month, although larvæ, mostly of *A. ramsayi* and *A. hyrcanus*, were still to be found. In February, although the breeding in association with this plant was at its lowest, with a record of 17 larvæ in seven observations, more adults were trapped than in November and December. The chief species breeding in association with *Pistia* during the winter months was *A. hyrcanus*.

Out of 2,309 larvæ collected in 213 observations during the period February 1936 to January 1937, *A. hyrcanus* (911) and *A. ramsayi* (759) were the principal species breeding in association with *Pistia*. Emergences also were comparatively high for these two species (282 and 189 respectively). *A. philippinensis* formed only a small proportion of the year's collection (larvæ 175, adults 31), and so far as can be judged from the data obtained, its maximum intensity was attained during June (larvæ 45 and adults 10 in ten observations). *Pistia* thus affords more breeding facilities to the innocuous species like *A. hyrcanus* and *A. ramsayi* than to *A. philippinensis*, the only important malarial vector in the area; this species, however, was breeding in association with *Pistia* throughout the year, except during February 1936.

#### 6. *Ipomœa reptans* Poir.

This plant, which is a perennial floating herb having smooth hollow stems and bunches of roots, was present in abundance in the tanks throughout almost the entire year. Thirty-one observations were carried out in four tanks, two each in the villages Doharia (Tanks 4 and 6) and Guchuria (Tanks 2 and 3).



The observations were continued from February to July with a break in April, when the tank at Doharia (Tank 6) was partially cleared and *Spirogyra* appeared in it, as already mentioned. Observations were, therefore, concentrated on the two tanks in Guchuria from May onwards. There was another break during the period August to October, owing to heavy rains or dearth of the plant in pure culture. In November, observations were resumed in Doharia, when another tank (Tank 4) was studied until the end of December.

Anopheline breeding was not very intense in water associated with this plant, and the emergence of adults was still less frequent; in a year's work, including thirty-one observations, only 177 larvæ were obtained and 13 emergences recorded. Nine species, viz., *A. hyrcanus*, *A. barbirostris*, *A. subpictus*, *A. vagus*, *A. annularis*, *A. ramsayi*, *A. philippinensis*, *A. varuna* and *A. aconitus*, were breeding in association with this plant, amongst only 4 of which (*A. hyrcanus*, *A. subpictus*, *A. vagus* and *A. annularis*) emergences were recorded. The predominant species found breeding were *A. hyrcanus* (63 larvæ) and *A. annularis* (45 larvæ). Emergences recorded were one each of *A. annularis* and *A. vagus* (both in June), 5 of *A. subpictus* and 6 of *A. hyrcanus*. It is, therefore, unlikely that much danger exists from the breeding of anophelines in ponds where *Ipomoea* is the only aquatic plant present.

#### 7. *Lemna minor* L.

*Lemna*, or the common duckweed, is a floating green-coloured flat-bodied plant. Eleven observations regarding this plant were made, nine in May 1936 and two in September. In Doharia, where observations on *Lemna* were first made, the plant appeared in abundance during the month of May. After this, within a short time it grew so thick in the tanks (Tanks 23 and 26) that the entire surface of the water was covered up and no anopheline larvæ could thrive under such conditions. Still later during the monsoon, *Pistia* appeared in the tanks in overwhelming quantity, so that no further observations on the association of anopheline breeding with *Lemna* were possible. It was not until September, when the plant was found once more in pure culture in a tank at Chakraghata (Tank 7), that observations could be resumed.

Only eleven mosquitoes, *A. subpictus* (8) and *A. vagus* (3), emerged in the trap-nets in May from water containing *Lemna*. These were also the only two species which were found as larvæ in such tanks in May (*A. subpictus* 31, *A. vagus* 35). In September, sparse breeding of *A. hyrcanus* (4 larvæ only in two observations) was recorded from a tank containing *Lemna* at Chakraghata. This tank contained *Limnanthemum cristatum* till May. After this, during the rains, *Pistia* prevailed in the tank for some time. The presence of *Lemna* restricts the breeding of anophelines and when grown sufficiently thick it is definitely inhibitory, the few larvæ present being chiefly those of innocuous species, such as *A. subpictus* and *A. vagus*.

Most authors agree that the presence of a thick belt of *Lemna* in a tank or pond is inhibitory to mosquito breeding (Iyengar and Sur, 1928; Covell, 1940; Sen, 1936; Johnson, 1903, quoted by Howard, Dyar and Knab, 1912; Boyd and Aris, 1929). Bentley (1910), however, considered that *Lemna* had no value in the prevention of mosquito breeding in Bombay. He was probably not dealing with tanks thickly covered with *Lemna*, since it is not unusual to find larvæ in the open spaces when the plant does not form a continuous layer over a sheet of water.



8. *Limnanthemum cristatum* Griseb.

This plant has orbicular floating leaves with small white flowers and is rooted in the mud, growing practically throughout the year in the area under observation. On the approach of the rainy season, other aquatic plants appeared, so that observations had to be suspended after June. Forty-three observations on the breeding and emergence of anophelines in relation to this plant were carried out in four tanks, one in each of the four villages, Doharia (Tank 18), Chakraghata (Tank 7), Guchuria (Tank 3) and Chandnagar (Tank 1) during 5 months, February to June 1936.

Nine species, *A. hyrcanus*, *A. barbirostris*, *A. ramsayi*, *A. annularis*, *A. philippinensis*, *A. pallidus*, *A. jamesi*, *A. subpictus* and *A. vagus*, were found breeding in association with *Limnanthemum*. All these species emerged as adults except *A. barbirostris*, *A. jamesi* and *A. pallidus*, larvæ of which were present in extremely scanty numbers. Breeding of *A. jamesi* (3 larvæ) was recorded in June, whereas larvæ of the other two species, *A. pallidus* (3) and *A. barbirostris* (4), were found in March and April. One adult specimen of *A. ramsayi* was captured in a trap over this vegetation during February, although larvæ of the species were not recorded during the month. The chief species found breeding was *A. annularis* (248 larvæ), followed by *A. philippinensis* (74) and *A. hyrcanus* (70). The maximum breeding, as also the maximum emergences, occurred during June.

*Limnanthemum cristatum* appeared to afford good breeding conditions for a large range of anophelines, and a fair number of emergences of adult mosquitoes can be expected from tanks with this vegetation.

9. *Najas foveolata* A. Br.

This is a slender aquatic submerged plant with branched stems and linear leaves. It appeared as a dark felted layer at the bottom of a tank in Doharia (Tank 10) in the rainy season, and it was only in winter, when the water subsided, that the plants projected above the surface. Observations (25 in number) were, therefore, carried out during December and January. Towards the end of January, *Lemna* also appeared.

Although as many as nine species (*A. hyrcanus*, *A. barbirostris*, *A. ramsayi*, *A. annularis*, *A. philippinensis*, *A. pallidus*, *A. subpictus*, *A. varuna* and *A. aconitus*) were breeding in association with the plant, 409 larvæ being collected, only three species, *A. hyrcanus* (6), *A. annularis* (5) and *A. philippinensis* (1), were taken as adults in the trap-nets during December, whilst in January, although 119 larvæ were collected, no emergences were recorded. *A. annularis* (175 larvæ) and *A. hyrcanus* (121) were the commonest species associated with this plant, followed by *A. pallidus* (49) and *A. philippinensis* (24). Thus, the presence of *Najas* may be associated with the breeding of several species of Anopheles, although the chances for emergence of the various species are not very pronounced in such places.

10. *Ottelia alismoides* Pers.

This is a submerged aquatic herb, rooted and succulent; leaves roundish or oblong and long-petioled and may float on the water surface. It grew luxuriantly during the rainy season and remained submerged. In winter, when the water-level fell, some of the leaves appeared on the surface of water. The



association of anophelines with this plant was, therefore, studied in December 1936 and January 1937; sixteen observations being made in Doharia in a tank which previously contained *Pistia* (Tank 19).

The species found breeding in association with *Ottelia* were *A. hyrcanus*, *A. barbirostris*, *A. annularis*, *A. philippinensis*, *A. pallidus* and *A. subpictus*, of which *A. annularis* was by far the most numerous (146 larvæ out of 238 being captured). *A. pallidus* was represented by 43 larvæ, *A. hyrcanus* by 39 and *A. philippinensis* by 7. Only five emergences of anopheline mosquitoes, two of *A. hyrcanus*, two of *A. annularis* and one of *A. philippinensis*, were recorded. It seems that, although there was nothing to prevent the breeding of anophelines in association with this plant, the adult output from such places was so scanty that it deserves no serious consideration as an aid to their production.

#### 11. *Hymenachne myurus* Beauv.

This is a tall aquatic grass with stout erect stems and long leaves with pointed tips. Thirteen observations regarding this plant were made during June, July and August in four tanks, two in Doharia (Tanks 16 and 26) and one each in Chakraghata (Tank 2) and Chandnagar (Tank 1). Of the two tanks at Doharia, one (Tank 26) had been under observation for *Ceratophyllum* in April and for *Lemna* in May. *Hymenachne* appeared in considerable quantity during June but was soon removed by the owner. In the other tank at Doharia (Tank 16), the grass appeared in August, but later it was associated with *Lemna* and *Azolla*. Similarly, the tank at Chakraghata was under observation for *Spirogyra* during February and March and for *Ceratophyllum* during the period April to June, while the tank at Chandnagar which contained *Hymenachne* in July was under observation for *Limnanthemum* during the period February to April. The grass in the tanks at Chakraghata and Chandnagar was associated with *Pistia* after July.

Altogether 71 larvæ of *A. hyrcanus*, *A. ramsayi*, *A. annularis*, *A. philippinensis*, *A. pallidus*, *A. subpictus* and *A. vagus* were collected from water containing this plant in thirteen observations from June to August, but only four adults, two of *A. ramsayi* and one each of *A. annularis* and *A. philippinensis*, emerged during July. Of the 71 larvæ, 30 were *A. hyrcanus*, 13 *A. annularis* and 12 *A. philippinensis*. It seems that water containing the grass may harbour the larvæ of several species of Anopheles, especially *A. hyrcanus*, but that only a small proportion of adults emerge.

#### 12. *Eichornia speciosa* Kunth.

This plant, commonly known as the water-hyacinth, is a perennial floating herb. The leaves have large swollen petioles and are arranged in the form of rosettes. The flowers are very attractive. Only one tank (Tank 25) containing this plant was kept under observation during April and May. With the advent of the rains, there was a dense growth of this plant and no anopheline larvæ were collected, whilst during the winter the water dried up as the tank was shallow. Only 17 larvæ, comprising the species *A. hyrcanus*, *A. ramsayi*, *A. annularis*, *A. subpictus* and *A. vagus*, were obtained from the area in six observations during April and May, while only one emergence each of *A. vagus* and *A. subpictus* was recorded, both in May. *A. subpictus* was not breeding in May when its emergence was recorded, but seven larvæ of the species were collected in April.



The association of water-hyacinth with anopheline incidence was also studied in Sonarpur, a comparatively healthy area with a spleen rate of approximately 10 per cent, during the period November 1934 to September 1935. This station is about 10 miles distant from Calcutta, on the southern section of the Eastern Bengal Railway. Here the plants were growing all the year round, and altogether 234 observations were carried out in four breeding places, of which one was a pond which dried up in March, while the others were tanks containing water throughout the year. All these breeding places were in a rice-growing region and were connected with the ricefields during the heavy rains.

In Sonarpur, *A. barbirostris*, *A. philippinensis*, *A. varuna* and *A. aconitus* were breeding, in addition to species recorded from Madhyamgram. Out of 2,901 larvæ collected, *A. aconitus* (1,000) was the most numerous, followed by *A. hyrcanus* (561) and *A. subpictus* (458). The maximum breeding of *A. hyrcanus* was in the cold weather, December and January, when thirty-nine observations yielded 227 larvæ; that of *A. aconitus* was in the spring, March and April, when sixty-three observations yielded 901 larvæ and of *A. subpictus* in June and July with the onset of the rains, when forty-four observations yielded 431 larvæ. The peak of breeding of *A. varuna* in association with water-hyacinth was, however, reached during September, when the breeding places were completely filled with rainwater; as many as 317 larvæ out of 370 of this species collected during the entire period, were recorded in September as the result of seventeen observations. The maximum breeding of *A. vagus* was reached in May, when twenty-two observations yielded 143 larvæ of this species out of a year's total of 285. Breeding of *A. philippinensis* was detected in July and August only; 11 larvæ of this species were recorded in thirty-eight examinations during these months.

Altogether 880 emergences, as revealed by trap collections, were recorded in 234 observations, including all the species known to be breeding in association with this plant except *A. ramsayi* and *A. philippinensis* which, however, were found as larvæ in scanty numbers only in such situations. The record of *A. philippinensis* in water-hyacinth tanks in Sonarpur was probably affected by their connection with the adjacent ricefields, which were known to favour the breeding of this species.

The intensity of emergences closely followed that of the larval prevalence. Thus, the maximum emergence of *A. hyrcanus* occurred in December and January (400 emergences out of 589, the total for the species for the year); that of *A. aconitus* in March and April, when all the 39 emergences of the species in association with this plant were observed; and that of *A. subpictus* during June and July when 30 emergences out of 54, the total for the entire period, were recorded, the remaining 24 emerging in November and December. Similarly, in September, *A. varuna* was almost the only species emerging, since out of 36 emergences during the month, 28, i.e., all those recorded during the year, belonged to this species. Another striking feature was that, while the breeding of anophelines continued, although in a reduced scale owing to heavy rains in August, the total number of anopheline larvæ collected in fifteen observations was only 163, there being no emergence under the trap-nets during the month except for one *A. hyrcanus*.

When water-hyacinth grows very thickly, as in Madhyamgram, the larvæ associated with it are few in number, and consist of harmless species only, whilst the emergence from such places is very poor indeed. The major adult output



from breeding places containing this plant, when not completely covering the water surface, as in Sonarpur, and in contiguity with rain-fed ricefields, was of *A. hyrcanus*, which constituted nearly 66 per cent of the total emergence of all species.

#### B. PREFERENTIAL PLANT ASSOCIATION WITH DIFFERENT ANOPHELINE MOSQUITOES.

The relative abundance of anopheline species in relation to the presence of different aquatic plants is shown in Table III. *A. hyrcanus* and *A. subpictus* were of general distribution and were associated with every kind of aquatic vegetation in the areas studied, although the incidence of the species differed to a great extent in different cases. Thus, the largest number of larvæ of *A. hyrcanus* was obtained in association with *Pistia* and *Najas*, whilst this species was poorly represented in company with *Hydrilla* and *Ceratophyllum*. *A. subpictus*, on the other hand, showed the most frequent association with *Spirogyra*, next in order came *Lemna* when not growing thickly, and *Eichornia*. The association of *A. subpictus* with other plants was very poor. *A. annularis*, also of common occurrence in that area, showed a closer association with *Hydrilla* and *Ceratophyllum*.

*A. ramsayi* has a strong preference for *Pistia*, almost the entire catch of the species originating from waters containing this plant. *A. philippinensis*, the only natural transmitter of malaria in the area, showed a preference for *Spirogyra*, *Utricularia* and *Limnanthemum*, *Pistia* standing very low in the list of plants with which this species was found associated. This species has been reported as having a close association with *Pistia stratiotes* in the Philippine Islands by Mieldazis (1930), but the present study shows that in Bengal it has such a wide range of association that *Pistia* alone cannot be credited with a close relationship. That *Pistia* does not afford an infallible breeding ground for *A. philippinensis* has already been reported by Krishnan (1940). *A. pallidus* is intimately associated with *Utricularia*, *Ottelia* and *Najas* coming next. The destructive action of *Utricularia* may account for the virtual absence of emergence of this species as evidenced by the trap-net catches.

*Eichornia*, under observation at Sonarpur, exhibited a preferential association with *A. aconitus*. *A. jamesi*, which occurred sparsely in this area, was associated with *Pistia*, *Ceratophyllum*, *Utricularia* and *Limnanthemum*, more or less to the same extent. *A. varuna* was plentiful in association with *Eichornia* at Sonarpur, and was associated with *Najas*, *Ipomæa* and *Pistia* at Madhyamgram. The solitary instance of *A. culicifacies* breeding was from a tank containing *Ceratophyllum*, although its emergence was recorded from a tank containing *Spirogyra*. *A. barbirostris* seems to have a dislike for *Utricularia*, *Hydrilla*, *Ceratophyllum* and *Hymenachne*, since the species was not breeding in association with these plants. It should be noted, however, that the observations regarding *A. barbirostris*, *A. varuna* and *A. jamesi* are based on insufficient data, since the species were sparsely represented in the area.

In Chart 1, it will be seen that when the number of species associated with each type of plant is considered, *Pistia* stands at the top, closely followed by *Eichornia*, *Spirogyra*, *Limnanthemum*, *Ipomæa* and *Najas*, but the emergence from breeding places containing the last two plants is very poor. When the plants studied are classified on the basis of the average number of individual anophelines associated with them, it is found that *Najas* and *Hydrilla* stand



TABLE III.

*Relative abundance of each anopheline species in relation to the different aquatic vegetations.*

Species.	<i>Pistia stratiotes.</i>	<i>Spirogyra</i> sp.	<i>Ceratophyllum demersum.</i>	<i>Utricularia flexuosa.</i>	<i>Hydrilla verticillata.</i>	<i>Ipomea aquatica.</i>	<i>Lemma minor.</i>	<i>Limnanthemum cristatum.</i>	<i>Najas foveolata.</i>	<i>Olethia alismoides.</i>	<i>Hymenachne myrtus.</i>	<i>Eleocharia speciosa.*</i>
<i>A. hyrcanus</i> Larvæ	42.7	20.0	nil	11.9	2.0	20.3	3.6	16.3	48.4	24.4	23.1	23.9
Adults	13.2	7.4	0.2	1.5	nil	1.9	nil	5.8	2.4	1.2	nil	24.7
<i>A. barbirostris</i> Larvæ	3.4	1.2	..	nil	..	3.2	..	0.9	7.2	0.6	..	3.7
Adults	2.5	nil	..	0.8	..	nil	..	nil	nil	nil	..	5.8
<i>A. ramsayi</i> Larvæ	35.6	0.2	nil	1.9	..	1.0	..	2.3	1.6	..	5.4	1.7
Adults	8.9	5.7	1.7	nil	..	nil	..	0.9	nil	..	1.5	nil
<i>A. annularis</i> Larvæ	12.6	35.5	99.4	62.9	146.3	14.5	..	57.6	70.0	91.2	10.0	3.8
Adults	4.9	9.0	54.1	8.5	31.0	0.3	..	8.6	2.0	1.2	0.8	0.2
<i>A. philippinensis</i> Larvæ	8.2	29.3	3.7	21.1	10.0	1.3	..	17.2	9.6	4.4	9.2	0.5
Adults	1.4	5.9	1.7	1.7	1.0	nil	..	3.2	0.4	0.6	0.8	nil



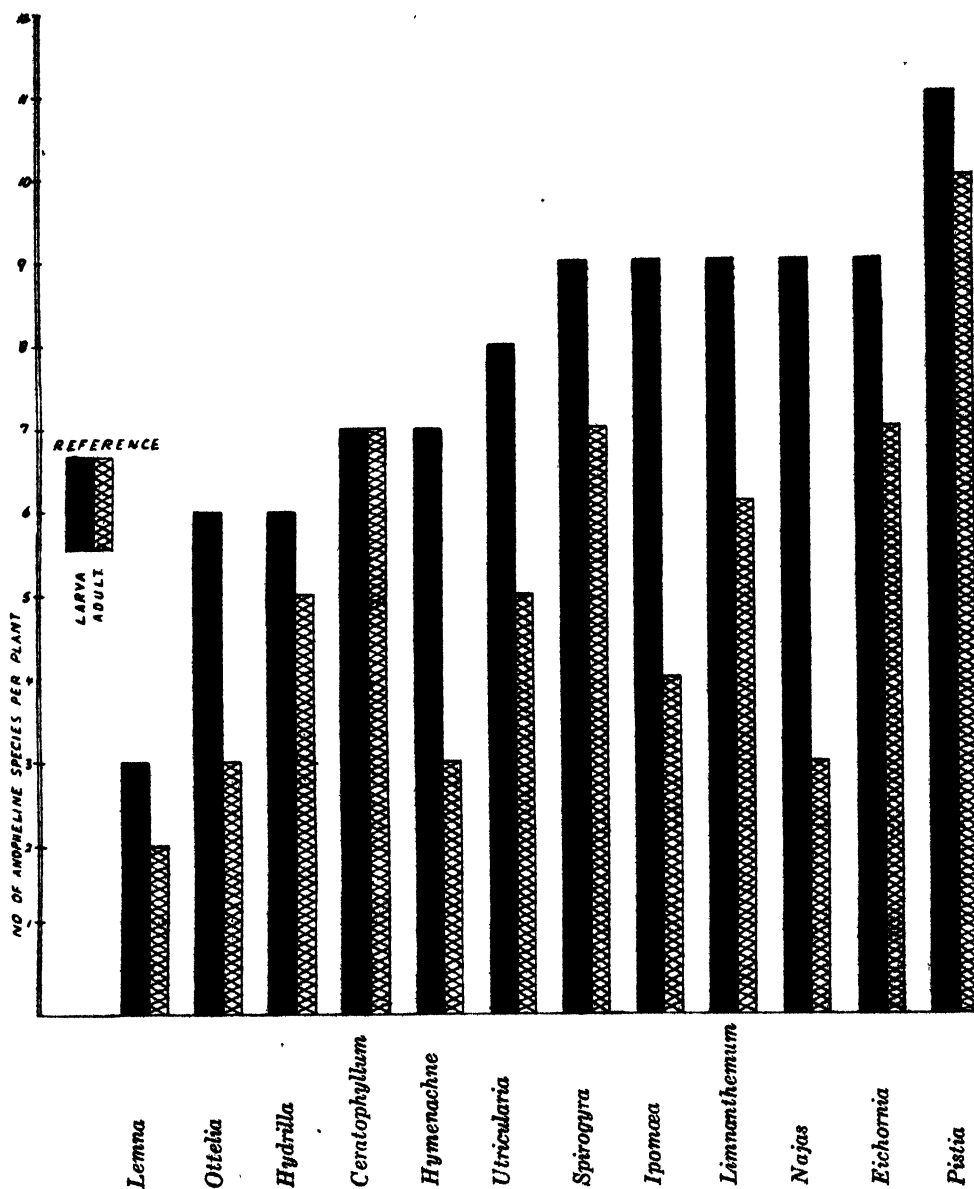
<i>A. pallidus</i>	Larvæ	0.7	1.0	0.2	52.7	2.0	..	..	0.7	19.6	26.9	0.8	..
	Adults	nil	nil	nil	0.2	nil	..	..	nil	nil	nil	nil	..
<i>A. jamesi</i>	Larvæ	0.4	..	0.9	0.4	..	..	..	0.7	..	..	..	..
	Adults	0.1	..	0.2	nil	..	..	..	nil	..	..	..	..
<i>A. subpictus</i>	Larvæ	1.3	33.6	6.6	0.4	1.3	8.7	28.1	9.1	0.4	1.2	2.3	19.5
	Adults	0.5	18.5	1.3	nil	2.3	1.6	7.2	2.3	nil	nil	nil	2.3
<i>A. vagus</i>	Larvæ	0.7	11.4	3.4	0.2	1.3	3.8	31.8	2.1	..	..	3.8	12.2
	Adults	0.4	3.1	0.3	nil	0.3	0.3	2.7	1.1	..	..	nil	1.7
<i>A. varuna</i>	Larvæ	2.1	..	..	..	..	2.2	..	..	6.4	..	..	15.8
	Adults	0.2	..	..	..	..	nil	..	..	nil	..	..	1.2
<i>A. aconitus</i>	Larvæ	0.5	0.2	..	..	..	2.0	..	..	0.4	..	..	42.7
	Adults	0.04	nil	..	..	..	nil	..	..	nil	..	..	1.6
<i>A. culicifacies</i>	Larvæ	..	nil	0.3	..	..	..	..	..	..	..	..	..
	Adults	..	0.2	nil	..	..	..	..	..	..	..	..	..

\* The data given under this plant are based on Sonarpur observations.

Note.—The figures represent the numeric abundance of Anopheles per ten observations.



CHART 1.

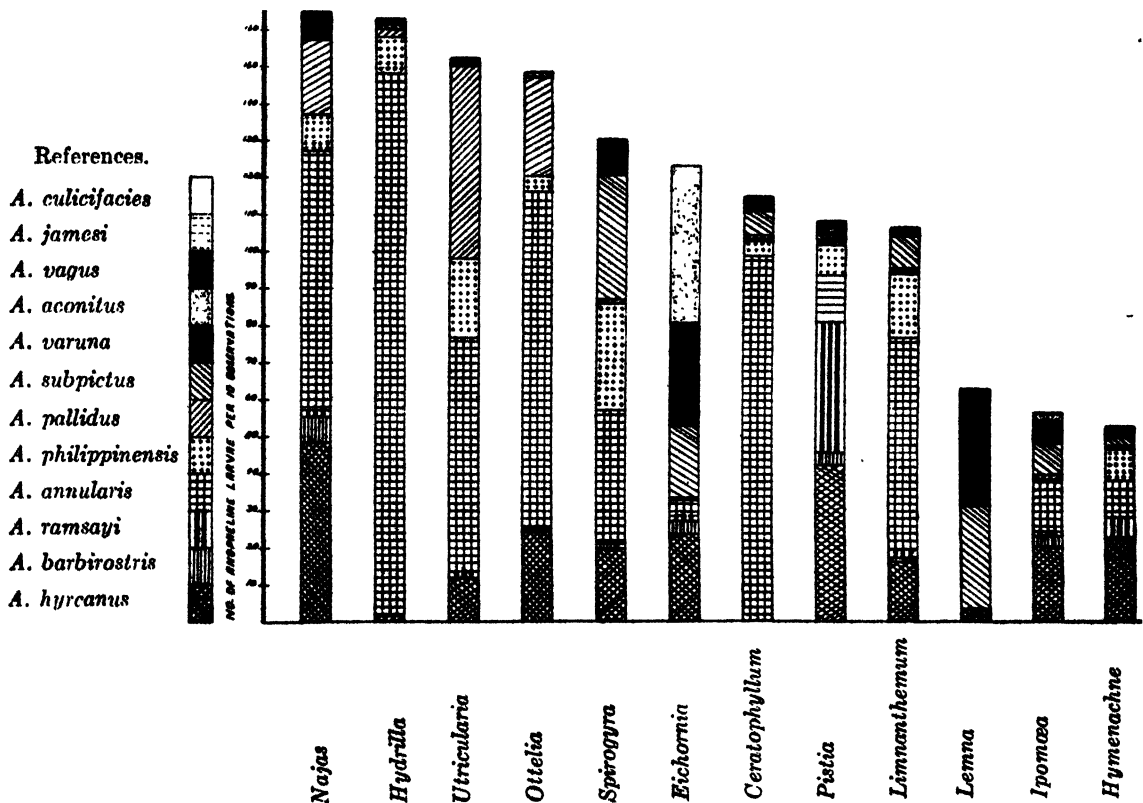


at the top with 164 and 163 individuals per ten observations respectively (Chart 2). Thus, only *Najas* shows a consistently strong association with anophelines in their larval stage, both in regard to the number of species and average number of individuals, although the emergence of mosquitoes from such breeding places is very poor, while the emergences from those containing



*Hydrilla* are fairly numerous. The studies of Kreckler (1930) on the animal population of submerged aquatic plants, however, show that the animal population associated with *Najas* plants stands very low indeed, being second from bottom, although with regard to the number of genera representing the fauna, the plant was found to occupy the second highest place among the plants examined.

CHART 2.



When both the breeding and emergence rates of the various anophelines recorded in relation to the different aquatic vegetations are considered together, it becomes clear that there are certain plants such as *Pistia*, *Spirogyra*, *Najas* and *Limnanthemum* which are found in breeding places containing larvæ of almost the entire range of anophelines existing in the area. But when the record of emergences from such waters is studied, it is evident that, whilst there are certain plants such as *Pistia* and *Ceratophyllum* whose presence is associated with the emergence of practically as many species as there were in larval stages, there are others such as *Najas*, *Ottelia*, *Hymenachne* and *Ipomoea* which appear to exert a harmful influence on the proportion of anopheline species which attain the adult stage.



TABLE  
*Anopheline incidence each month*

	<i>A. hyrcanus.</i>		<i>A. barbirostris.</i>		<i>A. ramsayi.</i>		<i>A. annularis.</i>		<i>A. philippinensis.</i>		<i>A. pallidus.</i>	
	Larvæ.	Adults.	Larvæ.	Adults.	Larvæ.	Adults.	Larvæ.	Adults.	Larvæ.	Adults.	Larvæ.	Adults.
February 1936 ..	59 22.9	27 10.4	4 1.5	2 0.8	3 1.1	4 1.5	19 7.3	10 3.9	45 17.3	10 3.9	3 1.1	.. ..
March ..	152 24.9	86 16.9	14 2.7	6 1.2	9 1.7	25 4.9	121 23.7	25 4.9	73 14.3	17 3.3	3 0.6	.. ..
April ..	38 7.03	6 1.1	34 6.3	12 2.2	7 1.3	10 1.9	328 60.7	82 15.2	36 6.6	7 1.3	2 0.3	.. ..
May ..	5 0.8	4 0.6	.. ..	.. ..	1 0.1	5 0.8	586 93.0	268 42.5	15 2.3	7 1.1	.. ..	.. ..
June ..	82 13.4	46 7.5	4 0.6	.. ..	73 11.9	11 1.8	521 85.4	154 25.2	125 20.5	22 3.6	.. ..	.. ..
July ..	96 16.9	36 6.3	1 0.1	5 0.8	441 77.3	123 21.5	103 18.0	22 3.9	99 17.3	10 1.7	8 1.4	.. ..
August ..	108 43.2	44 17.6	.. ..	.. ..	34 13.6	10 4.0	14 5.6	10 4.0	17 6.8	2 0.8	1 0.4	.. ..
September ..	206 47.9	55 12.9	17 3.9	28 6.5	20 4.6	22 5.1	23 5.3	11 2.5	13 3.0	2 0.4	1 0.2	.. ..
October ..	72 42.3	26 15.3	.. ..	.. ..	3 1.8	.. ..	1 0.6	3 1.8	9 5.3	2 1.2	.. ..	.. ..
November ..	246 58.5	12 2.9	15 3.5	1 0.2	145 34.5	23 5.4	19 4.5	.. ..	24 5.7	2 0.4	39 9.3	.. ..
December ..	218 44.5	15 3.4	8 1.8	.. ..	16 3.6	.. ..	304 69.1	10 2.2	86 19.5	12 2.7	210 47.7	1 0.2
January 1937 ..	106 21.2	3 0.6	16 3.8	4 0.8	42 8.4	.. ..	273 54.6	13 2.6	26 5.2	.. ..	103 26.6	.. ..
Total entire period	1,388	360	113	58	794	233	2,312	608	568	93	370	1
Average per ten observations.	26.0	6.7	2.1	1.1	14.9	4.3	43.3	11.4	10.6	1.7	6.9	0.1

*Note.*—Figures in the top row against a month denote the total catches during a month,



## IV.

in all types of vegetations.

<i>A. jamei.</i>		<i>A. subpictus.</i>		<i>A. vagus.</i>		<i>A. varuna.</i>		<i>A. aconitus.</i>		<i>A. culicifacies.</i>		Total larvæ all species.	Total adults all species.	Number of observations carried out.
Larvæ.	Adults.	Larvæ.	Adults.	Larvæ.	Adults.	Larvæ.	Adults.	Larvæ.	Adults.	Larvæ.	Adults.			
..	..	2	..	..	..	..	..	..	..	..	..	135	53	26
..	..	0.8	..	..	..	..	..	..	..	..	..			
..	..	6	2	..	..	..	..	..	..	..	..	378	161	51
..	..	1.2	0.4	..	..	..	..	..	..	..	..			
..	..	209	90	75	20	..	..	1	..	..	1	730	228	54
..	..	38.7	16.6	1.4	3.7	..	..	0.2	..	..	0.2			
7	1	59	21	49	7	..	..	..	..	2	..	724	313	63
1.1	0.1	9.3	3.3	7.7	1.1	..	..	..	..	0.3	..			
8	2	28	4	23	6	1	..	2	..	..	..	867	245	61
1.3	0.3	4.6	0.6	3.7	0.9	0.1	..	0.3	..	..	..			
1	..	16	1	..	..	..	..	..	..	..	..	765	197	57
0.1	..	2.8	0.1	..	..	..	..	..	..	..	..			
2	..	..	..	5	..	..	..	1	..	..	..	182	66	25
0.8	..	..	..	2.0	..	..	..	0.4	..	..	..			
..	..	..	..	..	3	30	2	..	1	..	..	310	124	43
..	..	..	..	..	0.7	6.9	0.4	..	0.2	..	..			
..	..	..	..	..	1	12	2	7	..	..	..	104	34	17
..	..	..	..	..	0.6	7.0	1.2	4.1	..	..	..			
..	..	2	..	..	..	4	..	3	..	..	..	497	30	42
..	..	0.4	..	..	..	0.9	..	0.7	..	..	..			
..	..	2	..	..	..	16	1	5	..	..	..	865	39	44
..	..	0.4	..	..	..	3.6	0.2	1.1	..	..	..			
..	..	2	..	1	..	4	..	..	..	..	..	573	20	50
..	..	0.4	..	0.2	..	0.8	..	..	..	..	..			
18	3	326	118	153	37	67	5	19	1	2	1	6,130	1,518	533
0.3	0.1	6.1	2.2	2.8	0.7	1.2	0.1	0.3	0.02	0.03	0.02	1,150	28.4	..

those in the bottom row denote catches per ten observations.



## C. ANOPHELINE BREEDING AND MOSQUITO OUTPUT IN ASSOCIATION WITH AQUATIC PLANTS.

The records of observations (Table IV) show that, although 2,312 larvæ of *A. annularis* and 1,388 of *A. hyrcanus* were collected, there were only 608 and 360 emergences respectively of these species during the corresponding period, whereas in the case of *A. subpictus* the number of larvæ collected was 326 and as many as 118 emergences were recorded, a considerably higher proportion. *A. ramsayi* occupied an intermediate position with a record of 794 larvæ and 233 emergences. The emergence rate of *A. philippinensis* was lower than that of any of the species mentioned above, since only 93 emergences were recorded, whilst 568 larvæ were collected. The number of emergences of *A. barbirostris* was 58, a comparatively high figure considering that only 113 larvæ of this species were collected during the corresponding period. There were only 37 emergences of *A. vagus* against a total collection of 153 larvæ. The emergences in the case of the other species encountered were so few that they deserve no special mention.

The proportion of emergences varies greatly at different seasons. For instance, the emergences of *A. ramsayi* in September were almost equal to the larval collections, while in March, April and May there was actually a preponderance of emergences over the numbers of larvæ recorded. On the other hand, during the cold weather, the emergences of the anopheline species in general were very low, notwithstanding the large number of larvæ found throughout the winter.

The observations above recorded show that the adult emergences of the various local anophelines are usually much less numerous than the larvæ. In rearing experiments, it has been shown that the mortality among pupæ was sometimes as much as 50 per cent, the average mortality rate in the laboratory being 22 per cent (Sen, 1935). Casualties among the adults emerging in nature are not likely to be less and the mortality among the larvæ is still higher. Matheson and Hinman (1929), in their experiments in hot weather with *Spirogyra*, *Oedogonium* and *Zygnema* in battery jars, found that the emergence of adults was about 10 per cent only in the case of culicine larvæ. In another experiment carried out in an aquarium tank without any vegetation, a similar emergence rate was again obtained.

## D. CHANGES IN THE ANOPHELINE FAUNA ACCOMPANYING THE CHANGES OF FLORA IN A TANK.

If the successive appearance of aquatic plants and their anopheline association in Tank 3 at Guchuria are traced throughout the year, it is seen that, when *Utricularia flexuosa* was present in May, a large number of *A. annularis* larvæ together with some *A. philippinensis*, *A. jamesi*, *A. subpictus*, and *A. vagus* larvæ were recorded. When the same tank contained *Limnanthemum cristatum* in June, larvæ of *A. annularis*, *A. philippinensis* and *A. hyrcanus* were present in large numbers, and a few of *A. vagus* were also collected, but *A. subpictus* was absent and *A. barbirostris* and *A. ramsayi*, which were not found before, now made their appearance. In July, when *Ipomœa aquatica* appeared in the tank, the only anopheline larvæ recorded were those of *A. ramsayi* and *A. annularis*.

At Chandnagar, Tank 1 contained larvæ of *A. hyrcanus*, *A. barbirostris*, *A. annularis*, *A. philippinensis* and *A. subpictus* from February to April in association with *Limnanthemum cristatum*. In July, when the tank contained



*Hymenachne myurus*, larvæ of *A. barbirostris* were not recorded, but those of *A. ramsayi* were now found to be present.

In Chakraghata, when *Spirogyra* was noticed in Tank 2 during February and March, larvæ of *A. hyrcanus*, *A. annularis*, *A. philippinensis* and, in small numbers, those of *A. subpictus*, *A. barbirostris*, *A. ramsayi* and *A. pallidus* were recorded. The same tank, when studied for *Ceratophyllum demersum* from April to June, contained larvæ of two additional species, *A. vagus* and *A. jamesi*, while *A. hyrcanus*, *A. barbirostris* and *A. ramsayi* were now absent. In July, however, when *Hymenachne myurus* was observed, *A. hyrcanus* and *A. ramsayi* were again recorded in addition to the three common species *A. annularis*, *A. philippinensis* and *A. subpictus*, but there were now no larvæ of *A. barbirostris*, *A. vagus* or *A. jamesi*. In another tank, at Chakraghata (Tank 7), which contained *Limnanthemum cristatum* during April and May, the presence of *A. annularis*, *A. subpictus* and to a lesser extent of *A. hyrcanus*, *A. philippinensis* and *A. vagus* was recorded. But when *Lemna minor* appeared in considerable quantity during September, the only larvæ observed were a few specimens of *A. hyrcanus*.

From Doharia also a similar change-over of the anopheline fauna accompanied the change of vegetation in a tank. In this village, Tank 26, in which *Ceratophyllum demersum* was growing during April and May, contained larvæ of *A. annularis*, *A. subpictus*, *A. vagus* and in scanty numbers *A. jamesi*, *A. culicifacies* and *A. pallidus*. When *Lemna minor* appeared in the tank during the latter part of May, only *A. subpictus* and *A. vagus* were found breeding in it. When *Hymenachne myurus* was present in June, larvæ of *A. hyrcanus* and *A. ramsayi* were recorded, while both *A. subpictus* and *A. vagus* were absent. In a second tank at Doharia (Tank 6), which contained *Ipomœa aquatica* in February and March, only a few larvæ of *A. hyrcanus*, *A. barbirostris*, *A. philippinensis*, *A. annularis* and *A. subpictus* were recorded. Later on, when *Spirogyra* was present in the month of April, larvæ of *A. barbirostris* were no longer found, but those of two additional species, *A. vagus* and *A. aconitus*, were now present.

It can be deduced from the above analysis that the presence of *Spirogyra* in a tank is compatible with the breeding of *A. hyrcanus*, *A. annularis*, *A. philippinensis* and *A. subpictus*; that of *Hymenachne myurus* with *A. ramsayi* and also *A. hyrcanus*, *A. annularis* and *A. philippinensis*; and that of *Ceratophyllum demersum* with *A. vagus* and *A. jamesi* during the hot weather in addition to *A. annularis*, *A. philippinensis* and *A. subpictus*. Larvæ of the local anophelines are not found to any extent in association with *Ipomœa aquatica* and still less with *Lemna minor*. Variations in the anopheline fauna accompanying changes in the flora of a tank appear to be largely a seasonal phenomenon.

#### IV. DISCUSSION.

Several authors (Coggeshall, 1926; Senior White, 1928; Howland, 1930a, 1930b) have investigated the feeding habits and nutrition of mosquito larvæ and have studied the plankton and other aquatic plant food, especially the floating algae commonly associated with mosquito breeding places. *Spirogyra* has been shown by various workers (Coggeshall, *loc. cit.*; Senior White, *loc. cit.*; Hancock, 1930) to afford a good medium for the growth of anophelines. Smith (1914) observed that, when tadpoles began to feed on the *Spirogyra* in a pool



containing larvæ of *A. punctipennis*, the species disappeared owing to the absence of food and shelter. According to Lamborn (1922), the food of the larvæ consists entirely of algæ. In the opinion of Metz (1919), almost all green algæ are suitable for anopheline development, and the larvæ can grow as well in non-living organic food of vegetable nature. Purdy (1920), however, thinks that microscopic organisms or plankton provide nutrition for the larvæ, and that the blue-green algæ, when not growing thickly, favour mosquito breeding in ricefields of Arkansas. Hamlyn-Harris (1928) states that the presence of algæ (*Spirogyra nitida*) favours the appearance of *A. annulipes* in Queensland.

While accepting plankton as the chief source of food for anopheline larvæ, most of these authors agree that they have no choice in the selection of the plankton, but feed indiscriminately. In other words, the larvæ adapt themselves to the food supply of the water in which they find themselves (Coggeshall, *loc. cit.*). This is in agreement with the observations recorded here. It is not the algæ alone on which anopheline larvæ depend for their development, for in the absence of algæ they will grow with equal vigour in a bed of *Ceratophyllum*, *Pistia*, or *Hydrilla* sustaining various other forms of plankton.

According to Rudolfs and Lackey (1929), the specific substances which are either present in the water or are produced by the decomposition of vegetable matter, may be responsible for the growth of micro-organisms and consequently for the breeding of mosquitoes. The present study has shown that certain species of anophelines have a close association with certain types of aquatic vegetations which provide, or are associated with, the food organisms necessary for the larvæ. The associations of *A. ramsayi* with *Pistia*, *A. subpictus* with *Spirogyra*, and *A. aconitus* with *Eichornia* are instances which may be cited.

Hinman (1930) puts forward an entirely different view. He does not support the theory of plankton feeding as advocated by some of the earlier observers. Mosquito larvæ, according to him, utilize the organic material in solution in water, living organisms being unimportant in this respect, although Barber (1927) found in his ingenious rearing experiments of anophelines in different culture media that dead organic material is unsuitable as food for larvæ.

Another point that has been brought out by this study is that frequently species found to be breeding in a vegetation belt would not show any evidence of emergence, or vice versa. There are various possible explanations for such anomalies. Apart from the effect of predators and parasites present in a natural water, there is a possibility of contamination with extraneous larvæ from neighbouring vegetation belts in a tank, or the emergences not supported by larval catches may have as their source some unnoticed pupæ of the species already existing in the area. The same might have happened in the case of *A. ramsayi* emergence from tanks containing *Ceratophyllum* and *Hydrilla*, although no larvæ of this species were found.

Instances of species not emerging at all although their larvæ were found in association with certain plants, are afforded by *A. barbirostris*, *A. pallidus* and *A. aconitus*. In almost every case, the larval catches of the species concerned were very scanty, and it is possible that those caught included the survivors originating from a batch of eggs deposited by some chance breeders of the species, or, if some larvæ remained, that these were not sufficiently mature to emerge during the time when the traps were in position.



## V. SUMMARY.

1. Anopheline breeding and emergence in relation to the presence of various aquatic plants, *Spirogyra* sp., *Ceratophyllum demersum*, *Hydrilla verticillata*, *Utricularia flexuosa*, *Ipomœa reptans*, *Pistia stratiotes*, *Lemna minor*, *Limnanthemum cristatum*, *Najas joveolata*, *Ottelia alismoides*, *Hymenachne myurus* and *Eichornia speciosa*, have been studied in certain villages in Lower Bengal, where malaria is endemic.

2. Altogether 12 species of anophelines, *Anopheles hyrcanus* var. *nigerrimus*, *A. barbirostris*, *A. ramsayi*, *A. annularis*, *A. philippinensis*, *A. pallidus*, *A. jamesi*, *A. subpictus*, *A. vagus*, *A. varuna*, *A. aconitus* and *A. culicifacies*, bred and emerged from waters containing the various plants above mentioned.

3. The presence of *Pistia stratiotes*, *Ceratophyllum demersum* and *Spirogyra* sp. was compatible with heavy breeding of the several anophelines recorded. The proportion of adult emergences from tanks containing these plants was also high.

4. The emergence rate was very low in association with *Utricularia flexuosa*, *Ottelia alismoides*, *Najas joveolata* and *Hymenachne myurus*, although the numbers of anopheline larvæ found in such breeding places were by no means insignificant.

5. *Lemna minor*, when growing thickly, appeared to be detrimental to anopheline breeding; *A. subpictus* and *A. vagus* were the chief species associated with this type of vegetation.

6. *A. ramsayi* was almost exclusively associated with *Pistia stratiotes*. *A. hyrcanus* also appears to have a strong preference for breeding places containing this plant, while this species seems to avoid *Ceratophyllum* and *Hydrilla* where other vegetation is present.

7. Waters containing *Hydrilla verticillata*, and next to this *Ceratophyllum demersum*, were most frequently associated with larvæ of *A. annularis*.

8. Larvæ of *A. philippinensis* were recorded from water containing all the types of plants studied excepting *Lemna*, but the species was most frequently associated with *Spirogyra* sp. *A. subpictus* was also frequently associated with this type of vegetation.

9. Larvæ of *A. pallidus* were most frequently found in association with *Utricularia*. The virtual absence of emergence of the species in the area may be due to the destructive action of the plant on the aquatic stages of this species.

10. For waters containing *Eichornia speciosa*, *A. aconitus* showed a special predilection.

11. The proportion of emergences of the different anopheline species, as well as the number of larvæ, varied according to the types of aquatic vegetation present and the season of the year at which the observations were made.

12. With regard to the number of different anopheline species associated with each type of plant, *Pistia* stands first closely followed by *Eichornia*, *Spirogyra*, *Limnanthemum*, *Ipomœa* and *Najas*, but as regards larval density *Hydrilla* and *Najas* stand at the top of the list.



## ACKNOWLEDGMENTS.

The author wishes to express his gratitude to Dr. S. N. Sur, Assistant Director of Public Health, Bengal, for suggesting this problem to him, and for the facilities received from him during the progress of this work. His thanks are due to Field Assistant J. C. Biswas, for his assistance in recording the field observations, and to Dr. K. P. Biswas of the Royal Botanical Gardens, Calcutta, for helping with the identification of the aquatic plants referred to in this paper.

## REFERENCES.

- BARBER, M. A. (1927) .. Food organisms (in pure cultures) of anopheline larvæ. *U. S. Pub. Hlth. Rpts.*, **42**, 22, pp. 1494-1510.
- BENTLEY, C. A. (1910) .. The natural history of Bombay malaria. *Jour. Bomb. Nat. Hist. Soc.*, **20**, pp. 392-422.
- BOYD, M. F., and ARIS, F. W. (1929). A malaria survey of the island of Jamaica, B. W. I. *Amer. J. Trop. Med.*, **9**, 5, pp. 309-399.
- COGGESHALL, L. T. (1926) .. Relationship of plankton to anopheline larvæ. *Amer. J. Hyg.*, **6**, pp. 556-559.
- COVELL, G. (1940) .. Anti-mosquito measures with special reference to India. Health Bulletin No. 11, Ed. 5. Govt. of India Press, New Delhi.
- HAMLYN-HARRIS, R. (1928) .. The relation of certain algæ to breeding places of mosquitoes in Queensland. *Bull. Ent. Res.*, **18**, pp. 377-389.
- HANCOCK, G. L. R. (1930) .. Some records of Uganda mosquitoes and the ecological associations of their larvæ. *Bull. Soc. Roy. Ent. Egypte*, New Ser. No. 1, pp. 38-56.
- HINMAN, E. H. (1930) .. A study of the food of mosquito larvæ. *Amer. J. Hyg.*, **12**, pp. 238-270.
- HOWARD, L. O., DYAR, H. G., and KNAB, F. (1912). The mosquitoes of North and Central America and the West Indies. Volume I. A general consideration of mosquitoes, their habits and their relations to the human species. Carnegie Inst., Washington, p. 156; and pp. 417-421.
- HOWLAND, L. J. (1930a) .. The nutrition of mosquito larvæ with special reference to their algal food. *Bull. Ent. Res.*, **21**, pp. 431-439.
- Idem* (1930b) .. Bionomical investigation of English mosquito larvæ with special reference to their algal food. *J. Ecology*, **18**, pp. 81-125.
- IYENGAR, M. O. T., and SUR, P. (1928). Report of the field malaria observatory at Sonarpur during 1921-1925. Bengal Government Press, Calcutta.
- JOHNSON, H. P. (1903) .. A study of certain mosquitoes in New Jersey, and a statement of the 'Mosquito malaria theory'. Appendix A, Rept. Ent. Dept., New Jersey Agr. Coll. Exp. St. for 1902, pp. 559-593. Quoted by Howard, Dyar and Knab (1912).
- KRECKER, F. H. (1930) .. A comparative study of the animal population of certain submerged aquatic plants. *Ecology*, **20**, 4, pp. 553-562.
- KRISHNAN, K. V. (1940) .. Report of the Professor of Malariology and Rural Hygiene. Annual Report of the All-India Institute of Hygiene and Public Health, Calcutta, 1939, pp. 35-36.
- LAMBORN, W. A. (1922) .. The bionomics of some Malayan anophelines. *Bull. Ent. Res.*, **13**, pp. 129-149.



- MATHESON, R., and HINMAN, E. H. Further studies on Chara spp. and other aquatic plants in relation to mosquito breeding. *Amer. J. Trop. Med.*, **9**, 4, pp. 249-266.
- METZ, C. W. (1919) .. .. Observations on the food of anopheline larvæ. *U. S. Pub. Hlth. Repts.*, **34**, pp. 1783-1791.
- MIELDAZIS, J. J. (1930) .. .. Preferential breeding conditions of Anopheles in the Philippine Islands. *Phil. Jour. Sci.*, **41**, pp. 59-63.
- PURDY, W. C. (1920) .. .. Biological investigation of California ricefields relative to mosquito breeding. *U. S. A. Pub. Hlth. Repts.*, **35**, 2, pp. 2556-2570.
- RUDOLFS, W., and LACKEY, J. B. The composition of water and mosquito breeding. (1929). *Amer. J. Hyg.*, **9**, pp. 160-180.
- SEN, P. (1935) .. .. Observations on the emergence of anophelines. *Rec. Mal. Surv. Ind.*, **5**, pp. 159-171.
- Idem* (1936) .. .. Report of the Field Malaria Observatory at Sonarpur for the years 1932-1935. Bengal Govt. Press, Calcutta, p. 25.
- SENIOR WHITE, R. (1928) .. .. Algæ and the food of anopheline larvæ. *Ind. J. Med. Res.*, **15**, pp. 969-988.
- SMITH, CORA A. (1914) .. .. The development of *Anopheles punctipennis* Say. *Psyche*, **21**, pp. 1-19.







## THE UTILITY OF MALARIA PARASITE INDICES IN INFANTS IN THE STUDY OF MALARIA.

BY

D. K. VISWANATHAN, L.M.S., B.S.SC., M.P.H. (Johns Hopkins),  
(Research Officer, Assam Medical Research Society, Shillong).

[March 21, 1941.]

### INTRODUCTORY.

RUSSELL, SWEET and MENON (1939) have referred to the paucity of literature in India relating to the study of malaria parasite indices in infants, although such indices have been extensively used elsewhere. In the present paper, the results of a study of 1,697 blood slides from infants are analysed by different age-groups and by months during the period March 1940 to February 1941.

Definitions of certain terms used are given below :—

*Infants* include all children below 12 months of age. Russell *et al.* (*loc. cit.*) included in their studies children of 12 months of age and under. As the term infant in public health practice applies to children below 12 months of age, the latter definition is retained.

*Age-groups*.—Four class intervals are used in the analysis, viz., below 2 months, 2 to 3 months, 4 to 5 months and 6 months and over. In each group, the age is reckoned with reference to the date of birth and that on which the blood specimen is taken. The first group requires no explanation, the second includes infants at least 2 months but not yet 4 months old, the third those at least 4 months but not yet 6 months old, and the last those who are at least 6 months but not yet 12 months old.

*Infant months of exposure*.—As infants of different age-groups have been exposed to infection for different periods, the number of months of exposure is reckoned for each group by summing up the number of days of exposure for each infant (between the date of birth and date on which blood is taken) and dividing it by 30. Less than half an infant month of exposure in the quotient is left out of account, and more than half is taken as one more infant month of exposure.

*Infant malaria index*.—The term index is preferred to the more precise term rate on the same analogy as parasite index is used in malariometry in preference to parasite rate, since the degree of accuracy of the results of a single examination for parasites will be of a much lower order than, say, in the case



of spleens. *Infant malaria index* means the percentage of infants showing malaria parasites.

*Index of infection per infant month of exposure* means the number of infants showing parasites per 100 infant months of exposure.

#### AREAS IN WHICH EXAMINATIONS WERE MADE.

The figures analysed include the routine quarterly infant blood examinations made in the surveys undertaken by the Assam Medical Research Society in Namdang Tea Estate, Baragolai and Ledo Collieries, Dhelakhat and Limbuguri Tea Estates, and in addition the examination of infant slides received by the courtesy of Dr. F. C. McCombie from some of the tea gardens in his practice including Gillapukri, Rangagora, Dinjan, Digultarrang and Baghjan. Infants at least 4 months old were examined only in 6 out of the 12 months, and those below 4 months of age every month. All these areas are situated in Upper Assam. The epidemiology of malaria in one of these areas, viz., Limbuguri Tea Garden, has been described elsewhere (Viswanathan, 1941). There is a prolonged season of malarial incidence commencing from April, reaching its height in July-August which is maintained in September, October and November, with a tendency to a notch in some years in September and a second peak in October-November and showing a fall in December-January, the lowest level of incidence being reached in February-March. The average daily minimum temperature falls below 60°F. from about the middle of December to early in March. The seasonal prevalence of adult *A. minimus*, the local vector, is roughly parallel to the malarial incidence, except that it is still relatively higher in December and very rare in April. Infections among *A. minimus* were encountered from May to December, with a peak in October-November. The spleen rates among children 2 to 10 years of age was over 80 per cent and the parasite indices were about 46 per cent in January 1940. Almost identical findings were obtained in the three tea gardens under detailed survey (Limbuguri, Dhelakhat and Namdang), and, in view of the paucity of *A. minimus* in April, it was surmised in the preliminary report on malaria surveys in Namdang Tea Estate and Dhelakhat Tea Estate (Viswanathan, 1940) that the malaria cases met with in April probably represent in bulk relapses of the infections acquired in the previous year. In all the tea gardens under study, the mothers of the infants examined include many who have lived in the area from birth for about 15 to 40 years, and have borne the brunt of a prolonged malaria season every year.

#### RESULTS OF EXAMINATION.

##### 1. WHOLE SERIES.

(a) *Infant indices*.—In the whole series 393 out of 1,697 infants, or 23·2 per cent, showed infection as compared with a parasite index of 46 per cent in the children 2 to 10 years of age. The indices among each age-group were 6·0 per cent (below 2 months), 14·9 per cent (2 to 3 months), 32·4 per cent (4 to 5 months) and 47·9 per cent (6 months and over). Such a progressive rise in infant indices with increasing age has also been described by Russell *et al.* (*loc. cit.*) in their examinations in Hiriyyur and Bobbur in Mysore State, but they found no evidence of a similar behaviour in their Pattukkottai series. While pointing out that such findings would vary considerably with the degree of endemicity of the area and possibly with the so-called immunity of the mother, they have rightly drawn attention to the fact that the older infants have greater



chances of infection owing to their longer period of exposure. Malaria in Mysore is of a much longer standing than in Pattukkottai. The indices in each age-group in Upper Assam are very much more than in Mysore, owing obviously to the higher degree of endemicity of the former area.

(b) *Index of infection per cent of infant months of exposure.*—In order to determine whether the progressive increase in the infant malaria index with increasing age is due to the longer period of exposure of the older infants or to any immunity factor in the younger infants inherited from their mothers, the index of infection per cent of infant months of exposure was determined. These indices were 6.0 (below 2 months), 6.0 (2 to 3 months), 7.2 (4 to 5 months) and 5.9 (6 months and over). It would, therefore, seem that the progressive rise in infant indices in the older infants is primarily due to the longer period of exposure. It is, however, possible that, while the index of infection per cent of infant months of exposure shows no variations in the different age-groups of infants, it may show significant variations in the different seasons of the year with a cumulative effect of cancelling each other on summation.

## 2. INFANT INDEX BY MONTHS AND AGE-GROUPS.

Table I and Graph 1 show the infant indices by month and by age-groups.

The indices for all infants show three distinct modes, in April, July and November, respectively. The April mode is of the highest order in the infants at least 6 months old, of a smaller order in the infants 4 to 5 months old, and is entirely absent in the infants under 4 months. Indeed, in the infants 2 to 3 months of age, the lowest index is recorded in April, and in the infants below 2 months the index is nil in March, April and May. The occurrence of successive waves of malarial incidence with increasing amplitude has been described elsewhere under conditions of epidemic prevalence over a prolonged period (Viswanathan, 1936). Although, therefore, the three modes in April, July and November may be possibly due to increasing fresh infections at intervals to allow for fresh batches of mosquitoes getting infected and transmitting infection, it is surmised on other data, such as the results of mosquito dissections, that the incidence in April is principally due to relapses. The highest indices in the oldest infant group, who have passed through a longer period of malarial season in the previous year, and the almost insignificant incidence in the younger infants born since January lend support to such a hypothesis. Further, the group 2 to 3 months shows a rising index in May and June, while the indices among the infants at least 4 months old show a distinct fall in June, indicating a special predilection for relapses in the month of April. The July peak is almost entirely due to fresh infections, although in the oldest group a small part may be made up of relapses. The infants below 2 months of age show a very small index of 2.4 in July, compared with 18.4 in the group 2 to 3 months, 48.4 in the group 4 to 5 months and 53.8 in the group at least 6 months old. This may be due either to their shorter period of exposure or some degree of immunity inherited from their mothers. All the groups show the peak in November, but while in the case of the peak in July the older infants show relatively much higher indices, in the November peak the relative proportions are of a smaller order. In other words, the youngest group shows a peak in November several times more than that of July, while in the older infants the peak in November is slightly of a lower amplitude than that of July, despite the fact that in November the older infants have passed through a longer period



TABLE I.  
*Infant index by age-groups and months in hyperendemic areas in Upper Assam.*

Month.	BELOW 2 MONTHS.			2-3 MONTHS.			4-5 MONTHS.			6 MONTHS AND OVER.			TOTAL.	
	N. E.	N. P.	Index per cent	N. E.	N. P.	Index per cent	N. E.	N. P.	Index per cent	N. E.	N. P.	Index per cent	N. E.	Index per cent
March 1940	58	0	nil	31	1	3.2	..	..	..	..	..	..	..	..
April	58	0	nil	56	1	1.8	41	10	24.4	94	52	55.3	249	25.3
May	44	0	nil	54	4	7.4	..	..	..	..	..	..	..	..
June	44	1	2.3	44	6	13.6	32	6	19.0	28	9	32.1	148	14.9
July	41	1	2.4	49	9	18.4	31	15	48.4	106	57	53.8	227	36.1
August	51	1	2.0	22	2	9.0	..	..	..	..	..	..	..	..
September	56	5	8.9	34	7	20.6	23	6	26.1	89	31	34.8	202	24.3
October	45	6	13.3	48	7	14.6	..	..	..	..	..	..	..	..
November	61	11	18.0	73	22	30.1	31	13	41.9	121	66	54.5	286	39.2
December	42	6	14.3	47	10	21.3	15	6	40.0	40	14	35.0	144	25.0
January 1941	37	3	8.1	26	4	15.5	..	..	..	..	..	..	..	..
February	13	0	nil	22	1	4.5	..	..	..	..	..	..	..	..
Total	550	34	6.0	496	74	14.9	173	56	32.4	478	229	47.9	1,697	23.2

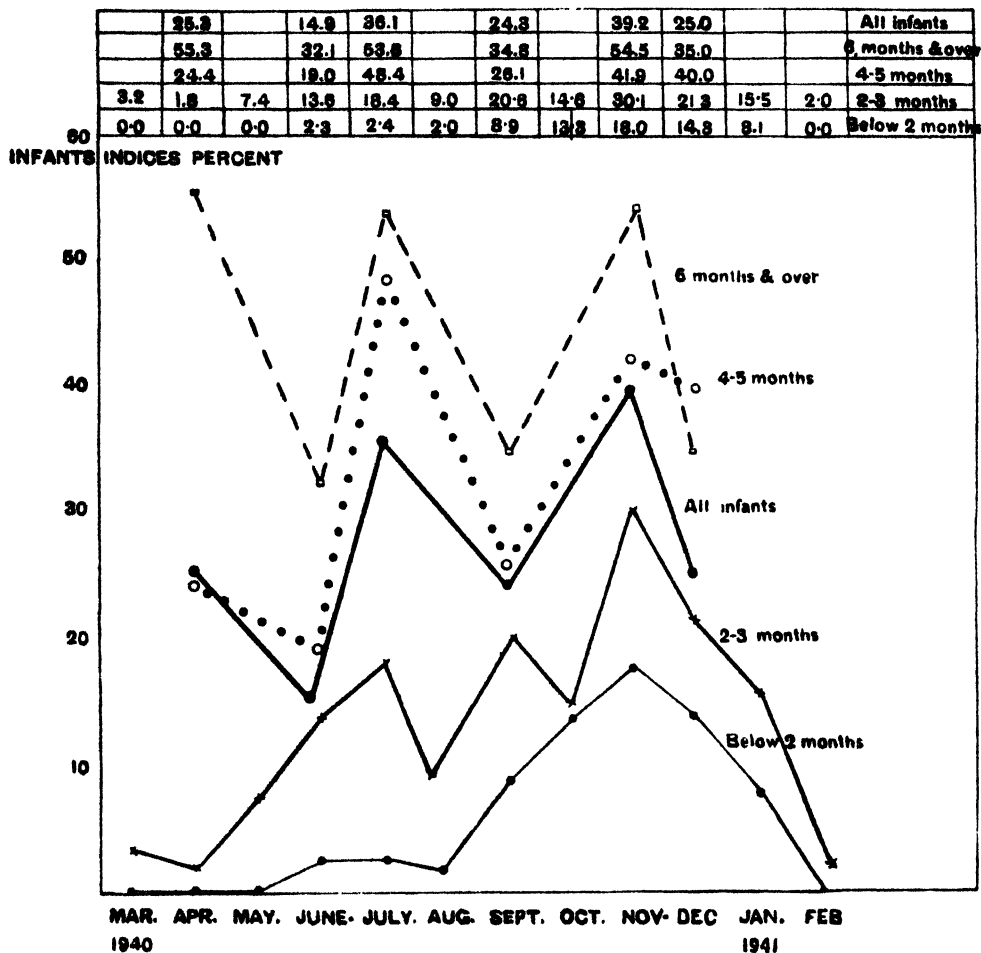
N. E. = Number of infants examined.

N. P. = Number with parasites.



GRAPH 1.

Infant indices by age-groups and months.  
Hyperendemic areas, Upper Assam: March 1940 to February 1941



of exposure in the malaria season than the younger infants. This leads to the conclusion that, at the earlier phase of transmission, the infants below 2 months of age show some evidence of inherited immunity which breaks down completely in the later phase.

### 3. INDEX OF INFECTION PER CENT OF INFANT MONTHS OF EXPOSURE.

Table II and Graph 2 show the index of infection per cent of infant months of exposure in each age-group and by months.

The indices for all infants show the three modes, referred to previously, in April, July and November. There is no need to consider the April peak any



TABLE II.  
Index of infection per cent of infant months of exposure. By age-groups and months.

Month.	BELOW 2 MONTHS.			2-3 MONTHS.			4-5 MONTHS.			6 MONTHS AND OVER.			TOTAL.	
	Infant months.	N. P.	Index per cent.	Infant months.	N. P.	Index per cent.	Infant months.	N. P.	Index per cent.	Infant months.	N. P.	Index per cent.	N. P.	Index per cent.
March 1940	58	0	nil	69	1	1.5	..	..	..	..	..	..	..	..
April	58	0	nil	131	1	0.8	186	10	5.4	845	52	6.2	63	5.2
May	44	0	nil	129	4	3.2	..	..	..	..	..	..	..	..
June	44	1	2.3	104	6	5.8	142	6	4.2	224	9	4.0	22	4.3
July	41	1	2.4	119	9	7.6	139	15	10.8	852	57	6.7	82	7.1
August	51	1	2.0	51	2	3.9	..	..	..	..	..	..	..	..
September	56	5	8.9	85	7	8.2	103	6	5.8	741	31	4.2	49	5.0
October	45	6	13.3	118	7	5.9	..	..	..	..	..	..	..	..
November	61	11	18.0	183	22	12.0	138	13	9.4	931	66	7.1	112	8.5
December	42	6	14.3	114	10	8.8	70	6	8.6	292	14	4.8	36	6.9
January 1941	37	3	8.1	68	4	5.9	..	..	..	..	..	..	..	..
February	13	0	nil	50	1	2.0	..	..	..	..	..	..	..	..
Total	550	34	6.0	1,241	74	6.0	778	56	7.2	3,885	229	5.9	393	6.09

N. P. = Number with parasites.



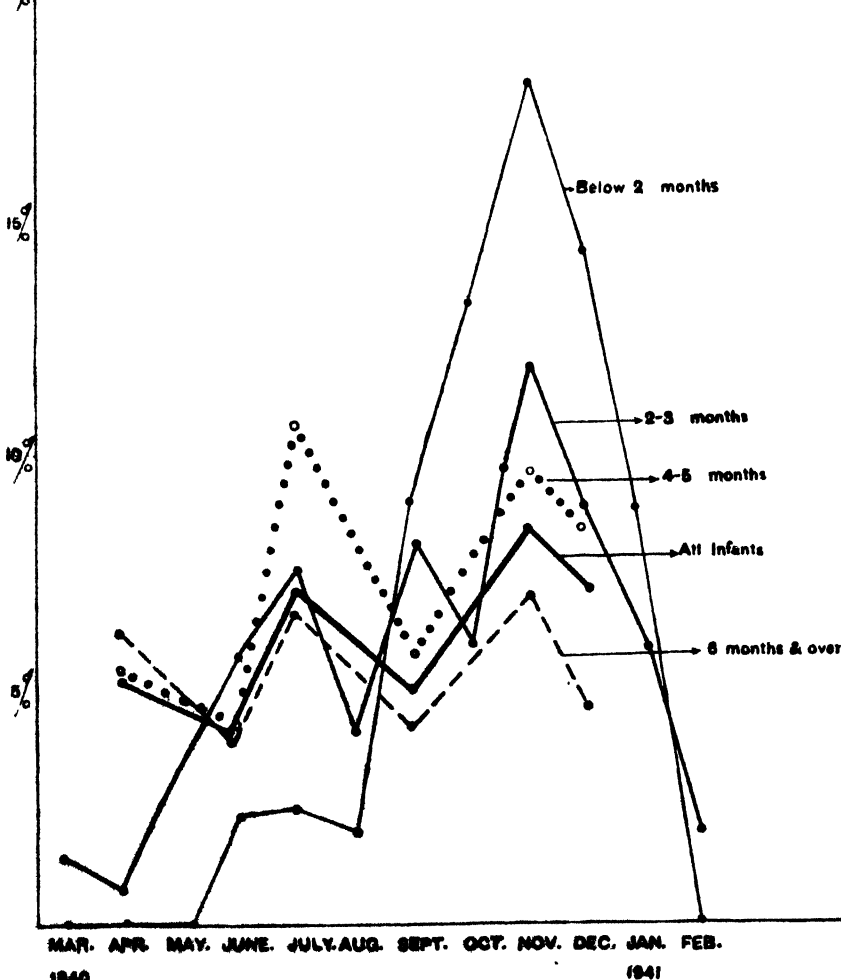
## GRAPH 2.

Index of infections per cent of infant months.

By age-groups and months.

Hyperendemic areas, Upper Assam: March 1940 to February 1941.

	5.2			4.3	7.1			5.0		8.5	8.9			All infants
	6.2			4.0	6.7			4.2		7.1	4.2			6 months & over
	5.4			4.2	10.2			5.8		9.4	8.6			4-5 months
1.5	0.8	3.2	5.8	7.6	3.9	8.2	5.9	12.0	8.8	5.9	2.0			2-3 months
0.0	0.0	0.0	2.3	2.4	2.0	8.9	12.3	18.0	14.3	8.1	0.0			Below 2 months

INDEX OF INFECTION PER CENT  
OF INFANTS MONTHS.



further. In the case of the July peak, it is interesting to note that the indices in the groups 4 to 5 months (10·8) and 2 to 3 months (7·6) are higher than in the older infants (6·7). The index in the youngest group is the lowest (2·4). There is very little difference in the degree of clothing among the infants, at all events, among the infants below 4 months of age. Hence the higher index in the group 2 to 3 months than in the group below 2 months points to the existence of some degree of immunity in the younger infants at the earlier phase of transmission. The infants at least 6 months old show a lower index than in the groups 4 to 5 months and 2 to 3 months respectively, in view of a much longer period of exposure, which includes a period during which little or no transmission occurs. The notch in August-September may be due either to a slight defect in mosquito output due to the flushing effects of the monsoon in July, or to the interval required for effective transmission in bulk after the first bout of infections, or to a combination of both factors. The November peak is inversely proportional to the age of the infant. It is 18·0 in the group below 2 months, 12·0 in the group 2 to 3 months, 9·4 in the group 4 to 5 months, and 7·1 in the group at least 6 months old. This may be due to three factors acting singly or in combination. In the first place, the probability of finding a parasite on a single examination in an infected infant below 2 months of age is several times more than in an infected infant at least 6 months old. In the former case, the infant will in all probability be passing through an acute primary attack, due to an infection acquired not more than 2 months back. In the latter case, the infant may have acquired its infection at any period from the commencement of the malarial season to the date of examination. By November, this interval is of several months' duration. There may be latent or low-grade infections which may be missed on a single examination. In the second place, the species and strains of parasites have to be considered. It may be that, with prolonged transmission, different strains of parasites are involved, against which not only may the feeble degree of inherited immunity in the youngest group be of no avail, but by reason of their extreme infancy they may actually exhibit an increased susceptibility. Whether the seasonal prevalence of the different species of malarial parasites has any bearing on this will be referred to later. In the third place, the infants below 2 months old who show the highest indices from October to December were born in the period August to October, while those who show low indices from May to August were born during the months March to June. The former group had a period of gestation from November-January to August-October and the latter from June-September to March-June. The intra-uterine life of the latter group corresponded with the period when the mothers were exposed to the risk of maximum transmission, viz., July to November. Hence, they were subject to a greater degree to the stimulus of fresh infections to increase their immunity quantum and transmit it to their progeny in a larger measure.

#### 4. SEASONAL INFECTION.

As the older infants are liable to relapse factor in April, and as the extremely young infants seem to have some degree of inherited immunity, the group 2 to 3 months will perhaps give the best idea of the quantum of seasonal infection. Only one infant in this group was found infected in March. This infant was born on January 11, 1940, and the blood was taken on March 16. He may have been inoculated at any period between these dates, but as even still younger infants were found to be infected in January as shown



later, it is possible that the infection occurred in this infant in that month, when he was less than a month old. In April, only one infant born on January 25, 1940, was found infected on April 16. In May, 4 infants were found infected, all of them born in February. This sharp rise in May probably indicates the sudden rise in hazard to fresh infections in that month, which is also corroborated by the finding of infective mosquitoes in that month. Such infections are also facilitated by an increase in the reservoirs of infection brought about by a sharp increase in relapses in April. Fresh infections continue to increase in June, show a diminution in July, rise again in August, show a slight notch in September (which may, however, be due to the small numbers examined in that month) and rise again in October and November, when the highest peak is reached. They fall in December and January, and in February only one infant in the group, born early in December, was found infected. The earliest age at which an infant was found infected was 15 days, an infant born on January 22, 1941, being found infected on February 5. The cumulative evidence presented above shows that fresh infections definitely occur from May to January inclusive. From February to April there is no evidence that fresh infections do occur. In April, the incidence of malaria is mainly due to relapses.

#### 5. SEASONAL INFECTION BY SPECIES OF PLASMODIA.

Table III shows the distribution of Plasmodia by species, seasons and age-groups.

Judging from the results of the entire series of infant blood examinations, *P. falciparum* was the most prevalent species at all seasons of the year. From November to April, however, infections with *P. falciparum* were relatively more numerous than from May to October. In the youngest group, 16 out of 34 infections were with *P. vivax*, 16 *P. falciparum* and 2 *P. malariae*. Similar proportions prevailed in the group 2 to 3 months (39, 38 and 1 respectively). In the older infants, *P. falciparum* was the more prevalent species. In the youngest group, the first infection with *P. falciparum* was met with in a child born on August 14, 1940, and examined on September 14. In the group 2 to 3 months of age, the first infection with *P. falciparum* was met with in a child born in March and examined in June. The highest infections with *P. vivax* relative to the total infections were met with in May to July among all infants. These findings are in conformity with the general seasonal prevalence of the species, viz., the prevalence of *P. vivax* in larger numbers in summer months and that of *P. falciparum* in the autumn. But the relatively lower proportion of *P. falciparum* infections among the younger infants indicates a greater degree of inherited immunity, if any, operating against that species. This immunity among the younger infants, however, breaks down in November to January, when they show as much relative prevalence of *P. falciparum* infections as the older infants.

#### DISCUSSION.

As stated by Russell *et al.* (*loc. cit.*), the study of infant indices is useful for the following purposes:—

1. To assess the quantum of infection in the course of a year.
2. To assess fairly accurately the effectiveness of measures for the control of malaria.



TABLE III.  
Distribution of Plasmodia by species, seasons and age-groups among infants in  
hyperendemic areas in Upper Assam.

Period of the year.	UNDER 2 MONTHS.			2-3 MONTHS.			4-5 MONTHS.			6 MONTHS AND OVER.			TOTAL.		
	P.v.	P.f.	P.m.	P.v.	P.f.	P.m.	P.v.	P.f.	P.m.	P.v.	P.f.	P.m.	P.v.	P.f.	P.m.
February-April ..	..	..	..	3	..	..	..	10	..	18	36	1	21	46	1
May-July ..	2	..	..	12	7	..	9	15	..	18	53	2	41	75	2
August-October ..	9	2	1	11	5	..	3	3	..	5	26	1	28	36	2
November-January	5	14	1	13	26	1	6	13	1	27	65	3	51	118	6
TOTAL ..	16	16	2	39	38	1	18	41	1	68	180	7	141	275	11
TOTAL ALL SPECIES ..	34			78			60			255			427		

Note.—There were only 393 infants who showed parasites but 34 among them showed mixed infections, hence the total number of infections is computed as 427.

P.v. = *P. vivax*.

P.f. = *P. falciparum*.

P.m. = *P. malarie*.



3. To determine, within limits, the actual season of transmission.
4. To determine whether different species of parasites have different transmission seasons.

To these may be added:—

5. To determine the prevalence of any immunity factor in the infant population.

These various uses would, however, depend on the method of collection and analysis of the data. A single annual examination of the infants would only give us a measure of the quantum of infection in the course of the preceding 12 months. Since most reports have to relate to a calendar or some other specified period of 12 months, the single examination should be made at the end of the malaria season within that period as far as can be ascertained, in order to throw light on the quantum of infection in the period under report. For instance, if in Upper Assam the period of report relates to a calendar year, an infant malaria index compiled from all infants in the month of April will only furnish a somewhat uncertain measure of the infection in the malaria season of the previous year, since the April parasite prevalence is largely made up of relapses. Hence, if only a single examination can be made it should be carried out in November to December, whether the report relates to a calendar year or to an official year from April to March. Such a single examination will help to determine the quantum of infection during the malaria season of the year under report. It will also be useful to determine the efficacy of the control measures carried out during the year far more precisely than spleen rates, especially in areas where malaria is long standing and has a prolonged season of transmission. The spleen rates in such areas do not usually show any significant reduction until some years have elapsed. Again, the spleen rates in children do not always reveal the true degree of endemicity if they are subject to regular and continuous quininization, as is the practice in many tea gardens, a practice which is commenced in many cases along with the institution of antilarval measures.

Examinations made twice a year may throw some light on the season of transmission if it is well defined. Russell *et al.* (*loc. cit.*) in pointing out the non-utility of analysing results of such bi-annual examinations by month of birth or age in months have resorted to the ingenious device of calculating the proportion of infants exposed in each month showing infection on a later examination. While this method of analysis makes the maximum use of the available data, they have themselves drawn attention to the fact that an infant exposed in a month and found infected later may have been infected at any period from birth up to the date of examination, i.e., either prior to, during, or subsequent to the month of exposure. Thus, infants infected prior or subsequent to the month of exposure will tend to exaggerate the hazard to infection in the particular month of exposure. Thus, some months of exposure having little hazard will show a higher rate of infection. But, since infants exposed and infected during the months of malaria transmission will tend to show infections to a greater extent on a later examination, their method of analysis may well be deemed to throw light, within limits, on the season of transmission.

Precise information regarding the season of transmission of all or individual species of *Plasmodium* and on the immunity factor can only be obtained by the method of analysis presented in this paper. Computation of



infant indices with respect only to the number of infants in each age-group will be fallacious, in that the period of exposure of infants of various ages in months is reckoned out of account. The index of infections per cent of infant months of exposure will give us more precise knowledge. But even here the odds may be loaded against the older infants, in that some period of non-malarial season may be added in computing the period of exposure resulting in a figure lower than the factual index. Again, the younger infants have a higher probability of showing infections on a single examination, as they will be in their acute primary attacks, while infections among older infants by reason of their having been acquired earlier or being latent or of low grade at the time of examination are less likely to be detected at a single examination. The relative paucity of infections among infants who were 5 months old or older at the beginning of the period of transmission described by Russell *et al.* (*loc. cit.*) may in part at least be due to this factor, especially when the total infant index in the community is as low as 6.2 per cent.

Bearing these limitations in mind, the results of the analysis presented in this paper show certain well-defined features.

1. *Season of transmission.*—From May to January active transmission takes place with two distinct peaks, one in July and the other in November. Any transmission that takes place from February to April is of a small order. While this is supported by other data relating to density of *A. minimus* adult infestation in the surveys in some of the tea gardens in the present group, Rice and Mohan (1936) have shown that the larval and adult output of *A. minimus* goes on unchecked throughout the cold months in a tea garden near Doom Dooma town. The meteorological conditions show very little variation between their experimental garden and those studied in the present report. There is one important difference, however, in that there is a perennial stream near their experimental garden, while there is no such perennial stream in the vicinity of Limbuguri Tea Garden. It is, therefore, possible that transmission may take place even in the cold months of February and March, provided there are suitable breeding grounds despite the low temperature in the cold season. The micro-climate in most of the coolie lines may be several degrees higher than the recorded temperature in shade. However, Rice and Mohan (*loc. cit.*) remark that the adult *A. minimus* captured in the cold weather consist of comparatively young females. They found two infections in nature, in one of their observation stations, towards the end of March, one with oöcysts and one with sporozoites, and in transmission experiments in the laboratory they likewise observed on March 31, two specimens showing infection, one with oöcysts and the other with sporozoites. In their large series of naturally-caught *A. minimus*, their total infection rates were 5.1 per cent in December, 2.0 per cent in January, 2.1 per cent in February, and 9.9 per cent in March; and their infectivity rates were 1.8 per cent in December, 0.3 per cent in January, 0.0 per cent in February, and 2.6 per cent in March. Although their work was carried out in an area exceptionally suited for static transmission, their own findings show no infectivity in February and little infectivity in January. There is one feature common to the malaria morbidity figures of all the dispensaries in the province, viz., the lowest level of incidence is reached in the months of February and March. Hence, the conclusion arrived at on the present study that February and March are the months of lowest inoculation seems well borne out, and is not inconsistent with the results of the study of Rice and Mohan (*loc. cit.*). In their own area too, although infections were found in those months, they were the lowest for the year. To the malarial



investigator, the behaviour of mosquitoes as determined by experimental study is of paramount importance. To the practical hygienist, the human response to such behaviour is of even greater importance. The present study, which deals with the later, shows that the degree of infection in February and March is of a very low order, and it is not inconsistent with the studies on the behaviour of the vector species. It has also been shown that the sharp rise in the curve of malaria incidence in April is principally made up of relapses.

2. *P. vivax* commences its period of transmission from about May and is most prevalent in May to July. *P. falciparum*, the more prevalent species at all times of the year, has an enhanced period of transmission commencing from June to July and reaching its height towards November.

3. Infants below 2 months of age seem to have some degree of inherited immunity from their mothers, which is manifested in the earlier phases of transmission but breaks down completely in the later phases. At first sight, this would seem to be due to such immunity being effective against *P. vivax* but ineffective against *P. falciparum*, which is more prevalent in the later phases. Such is not, however, the case, since *P. falciparum* is relatively less prevalent among the younger than the older infants. Actually, therefore, the inherited immunity is more effective against *P. falciparum*. Granting the existence of an inherited immunity, its quantum against the more malignant species is likely to be of a higher order in as much as the mothers represent the survivors of its more severe onslaughts.

The failure of this small quantum of inherited immunity in the youngest infants in the later phases of transmission, if it is not due to the species factor, may be due to the strain factor. In the later phases, more strains of Plasmodia may be transmitted against which it breaks down completely and by reason of their extreme infancy they suffer relatively more.

Some evidence is also furnished to account for this failure by reason of the mothers of the youngest infants born during the later phases having been exposed to a shorter period of the active malaria season. Mulligan, Somerville and Swaminath (1940), in their studies on the correlation of humoral and cellular agencies in the mechanism of defence against malaria, have shown that the value of homologous immune serum in the treatment of *P. knowlesi* infections in *rhesus* monkeys is enhanced by stimulation with prior infection with *P. cynomolgi*. It would, therefore, seem that even in mothers who have lived in the hyperendemic area for long and acquired some resistance, the stimulus of a further infection to their cellular mechanism of defence increases their quantum of humoral defence which alone they are in a position to transmit to their progeny.

Barber *et al.* (1936; 1937) and Clark (1937) quoted by Sinton (1939) noted a similar passive immunity which they suggest may have been transmitted from the mother to the offspring through the placenta or during the period of lactation.

4. On account of the possibility of the relapse factor in the older infants and of the immunity factor in the younger infants, the group 2 to 3 months of age is likely to give the best index of quantum of seasonal infection.



## SUMMARY.

1. The results of analysis of 1,697 blood examinations for a period of 12 months among infants in hyperendemic areas in Upper Assam where malaria is long standing are presented.

2. There is a well defined but prolonged season of malaria transmission from May to January inclusive, with a special predilection for relapses in April.

3. *P. vivax* has an earlier period of transmission from May and is most prevalent from May to July.

4. *P. falciparum*, the most prevalent species all through the year, is specially numerous from June to July and is most prevalent in the last quarter.

5. Infants below 2 months old seem to have some degree of inherited immunity from their mothers, which is more manifest against *P. falciparum* in the earlier phases of transmission and which completely breaks down towards the later phases.

6. In view of the relapse factor in the older infants and the immunity factor among the youngest infants, the group 2 to 3 months is likely to furnish the best evidence of the quantum of seasonal infection.

## REFERENCES.

- BARBER, M. A., MANDEKOS, A., and *Amer. J. Hyg.*, **24**, pp. 249-267.  
 RICE, J. B. (1936).  
     *Idem* (1937) .. .. *Ibid.*, **26**, pp. 175-185.  
 CLARK, H. C. (1937) .. .. *South Med. J.*, **30**, pp. 848-850.  
 MULLIGAN, H. W., SOMMERVILLE, T., *J. Mal. Inst. Ind.*, **3**, pp. 563-577.  
     and SWAMINATH, C. S. (1940).  
 RICE, E. M., and MOHAN, B. N. *Rec. Mal. Surv. Ind.*, **6**, pp. 557-594.  
     (1936).  
 RUSSELL, P. F., SWEET, W. C., and *J. Mal. Inst. Ind.*, **2**, pp. 439-455.  
     MENON, M. K. (1939).  
 SINTON, J. A. (1939) .. .. *Ibid.*, pp. 71-83.  
 VISWANATHAN, D. K. (1936) .. .. *Rec. Mal. Surv. Ind.*, **6**, pp. 239-271.  
     *Idem* (1940) .. .. Reports on preliminary malaria survey in Namdang  
   T. E. and Dhelakhat T. E. (MS.).  
     *Idem* (1941) .. .. *J. Mal. Inst. Ind.*, **4**, pp. 35-55.



### **ABSTRACT.**

## **THE INVESTIGATION OF THE HIGH INCIDENCE OF MALARIA IN MAWCHI MINES AREA, SITUATED IN KARENNI STATES, BURMA, AND THE MEASURES ADOPTED TO COMBAT AND CONTROL THE DISEASE.\***

BY

E. R. JAMES, M.R.C.S. (Eng.), L.R.C.P. (Lond.),  
(*Chief Medical Officer, Mawchi Mines*).

[December 23, 1940.]

THE area under investigation is situated in Bawlake State at an elevation ranging from 1,500 to 4,200 feet above sea level. The terrain is made up of steep hill sides, deeply cut by rocky ravines, through which flow a number of perennial and seasonal streams. Nine-tenths of the annual rainfall occurs during the south-east monsoon, i.e., from the middle of May to the end of September, the total yearly precipitation averaging from 60 to 75 inches.

Malaria was endemic at Mawchi prior to 1934, but it was only after the recent opening up of communications with other parts of the country that the disease assumed an epidemic character and began to exert a serious effect on the efficiency of the local labour forces.

\* A copy of the original manuscript has been placed in the Library of the Malaria Institute of India, Kasauli. This is available on loan to workers who wish to consult it.  
(*Editor.*)



Spleen rates recorded in various parts of the area in 1939 showed great variations as shown in the table.

TABLE

		Number of children examined.	Spleen rate per cent.
Mine camp	.. ..	60	0.5
Batavia camp	.. ..	133	2.3
13 camp	.. ..	104	4.8
Tek Bahadur	.. ..	27	7.2
Lokaloc	.. ..	110	11.8
Mill camp	.. ..	170	20.2
Yawthe Doe	.. ..	14	26.6
Mid camp	.. ..	6	50.0
Flume line village	.. ..	15	92.3

The author notes that the villages suffering most severely from malaria were those situated in close proximity to streams. Out of 356 positive blood examinations carried out in 1939, 256 infections with *Plasmodium vivax* were recorded, 99 with *P. falciparum* and 6 with *P. malariae*.

The following species of Anopheles have been identified :—

<i>A. maculatus</i>	<i>A. barbirostris</i>
<i>A. kochi</i>	<i>A. aitkeni</i>
<i>A. leucosphyrus</i>	<i>A. hyrcanus</i> var. <i>nigerrimus</i>
<i>A. minimus</i>	<i>A. vagus</i>
<i>A. culicifacies</i>	<i>A. annularis</i> .

*A. maculatus* is the most common species, and is considered to be the principal malaria carrier. It should be noted, however, that this has not yet been confirmed by dissection.

Antilarval measures include the flushing of streams by automatic siphon sluices, subsoil and contour drainage, canalization and stone-packing of streams, growing of shade-giving plants over streams and the systematic application of oil to breeding places by sprayers, drip-cans and booms. Indiscriminate clearing of jungle, which is likely to favour the breeding of *A. maculatus*, is prohibited.

It is claimed that these measures have already produced results of considerable value in reducing the local malaria incidence.

G. C.



***ERRATUM.***

*For 0.05 per cent read 0.5 per cent in line 3, page 47, Vol. IV, No. 1 (June 1941) of the  
Journal of the Malaria Institute of India.*







## SPLEEN AND PARASITE SURVEYS IN CEYLON.

BY

V. SIVALINGAM, M.R.C.P., D.T.M. & H.,  
*Medical Officer, Antimalaria Campaigns,*

AND

K. J. RUSTOMJEE, M.R.C.S., L.R.C.P., D.T.M., D.P.H.,  
*Superintendent, Antimalaria Campaigns.*  
(*Department of Medical and Sanitary Services, Ceylon.*)

[June 20, 1941.]

SINCE Dempster (1848) first pointed out the significance of the enlarged spleen as an indication of the malariousness of a country, the spleen rate has been utilized for the determination of the degree of malarial endemicity. Christophers and Khazan (1924) extended its usefulness by advocating the accurate measurement of the enlarged spleen. Schüffner (1919) demonstrated the value of correlated studies of splenic enlargements and parasite findings in a community.

### TECHNIQUE.

In our surveys no absolute measurements of splenic enlargements were made, but the enlarged spleens were placed in three categories : (i) palpable down to one finger's breadth below the costal margin; (ii) beyond one finger's breadth but not reaching the level of the umbilicus; and (iii) reaching the umbilical level or beyond this. In mapping out the areas, however, all degrees of enlargements have been classed together. The surveys were conducted among boys in schools throughout the island during the period February to March both in 1938 and 1939. The ages were from 5 to 14 years, with a few exceeding this range.

Contemporaneously thick and thin blood films on the same slide were prepared from 10 per cent of the children palpated for splenic enlargement during 1938 as advocated by Sinton (1925); and in 1939 this proportion was increased to 33 per cent in those parts of the island where the population was scanty, so as to secure a larger number of blood slides. The smears were stained, the thick with Giemsa and the thin with Leishman, and 100 microscopic



fields of the latter and 20 of the former were examined under a magnification of about 600 diameters.

The result obtained for each school was plotted independently on maps, after which the localities showing the same rates were connected together into iso-splenic and iso-parasitic areas.

The officers in the field performed the spleen examinations and secured the blood smears. The examination of the blood films and the analysis of the spleen results were carried out in our laboratories.

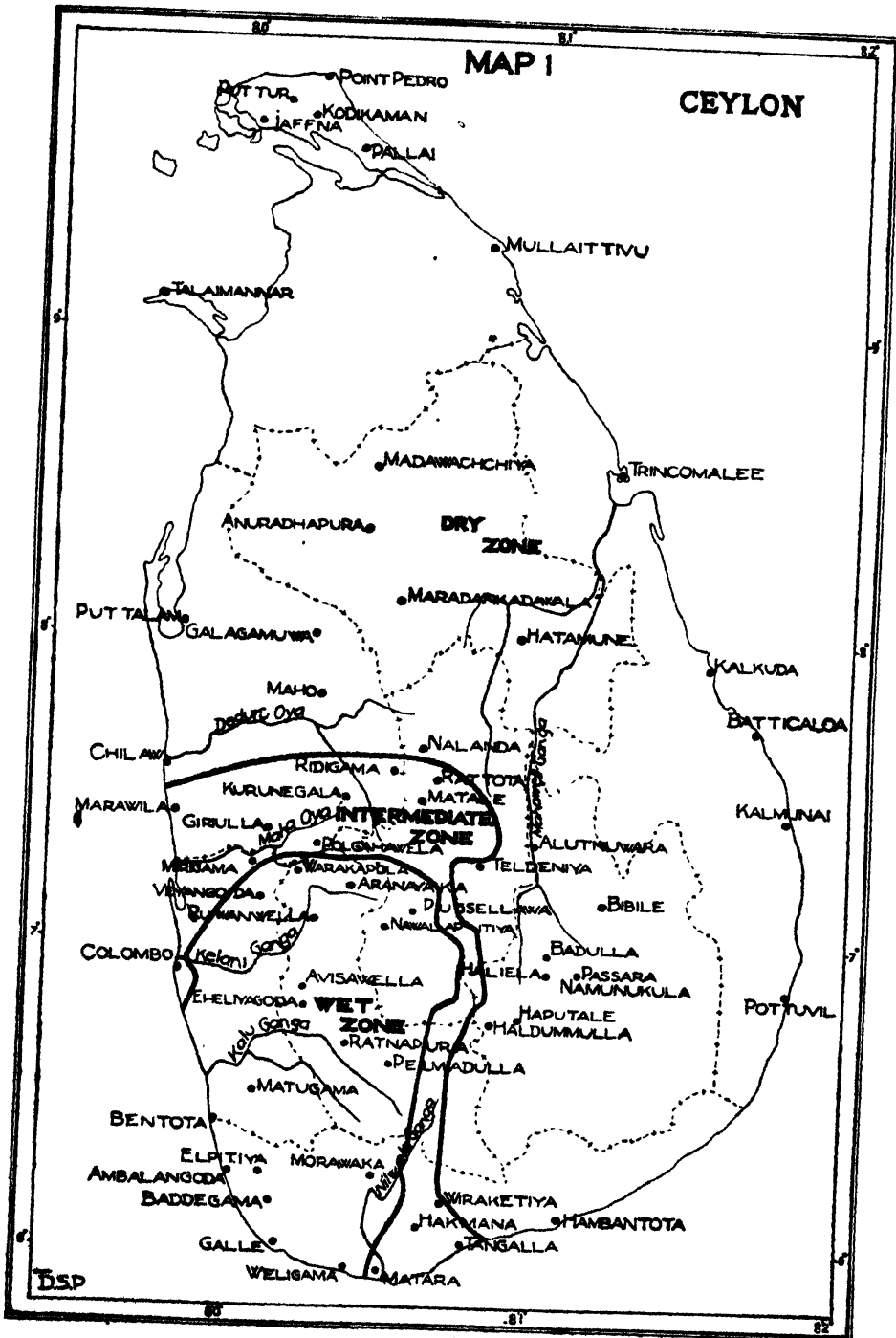
### PHYSICAL FEATURES AND CLIMATE.

The island is 270 miles long and 140 miles broad and is divided into nine provinces. According to the 1931 census its population was 5·3 millions. The south-central parts are mountainous, reaching to a height of about 7,000 feet, while the rest are flat and low-lying. Of the rivers, the important from malaria point of view are the Mahaweli Ganga, the Deduru Oya, the Maha Oya, Kelani Ganga, Kalu Ganga and Nilwala Ganga (Map 1). Rainfall depends on the two winds—the south-west and the north-east. The rainfall in the different parts of Ceylon during the south-west monsoon has a characteristic variation, and based on the amount of fall during that monsoon, the island has been divided into three zones, namely, the Wet Zone with over 40 inches of rain, the Intermediate Zone of 20 to 40 inches and the Dry Zone with less than 20 inches. The average temperature varies between 80°F. in the lowlands and 59°F. in the hill-country. The average relative humidity is of the order of 75 to 80 per cent.

### PREVIOUS WORK.

The first spleen census to be taken in Ceylon was organized by Dr. A. Perry, Principal Civil Medical Officer, in 1908. The subjects examined were children in schools, and young persons and children attending the Government dispensaries. The results were shown by provinces, and the spleen rate for the whole island was 34·05 per cent (Perry, 1909). Similar observations were made in each of the years 1909, 1912 to 1913, 1914, 1915 and 1916, and the rates per cent obtained for the whole island were 20·81, 25·74, 43·9 (Perry, 1910; 1914; 1915) and 33·8, 35·58 (Rutherford, 1916; 1917) respectively. In 1918 the malaria index was computed from the examination of school children (Rutherford, 1919). The number examined was 21,903 and the spleen rate was 21·4 per cent. Gunasekara (1913) examined 1,288 children between the ages of two and ten years in Kurunegala Town in September 1911, and recorded a spleen rate of 60·03 per cent. He found that quartan was the most prevalent parasite, the next in order being *P. vivax*, and that the malignant tertian parasite also occurred. James and Gunasekara (1913), from the examination of children in the villages in April 1913, recorded a spleen rate of 58 per cent (167 observations) and a parasite rate of 36 per cent (117 examinations) at Talaimannar. Quartan constituted 71 per cent, benign tertian 18 per cent and malignant tertian 10 per cent of the positive blood films. Bahr (1913) examined children under 14 years of age in Kurunegala Town, during the period February 18 to March 11, 1913. The spleen rate among 435 examined was 34·7 per cent and the parasite rate 10·5 per cent; quartan parasites were observed 33 times, benign tertian 12 times and malignant tertian (crescents) 3 times. James (1914) carried out observations in Jaffna and the northern ports during







August 11 to 21, 1913, and recorded in Jaffna and its environs a spleen rate of 3·3 per cent, in Puttur nil, in Point Pedro 2 per cent, in Pallai 60 per cent and in Kalmunai Spit 70 per cent. Of the positive blood films, quartan parasites were observed in 73 per cent, and benign tertian in 26 per cent.

Carter examined random samples from the inhabitants of all the more accessible towns and villages throughout the island between July 1921 and July 1922. For the most part school children under 12 years of age were examined, but in sparsely populated districts, village children and adults were included. The examination of 39,417 children yielded a spleen rate of 12·3 per cent. Out of 3,503 blood films examined, 8·5 per cent were positive for malaria parasites; benign tertian formed 77·4 per cent, malignant tertian 15·8 per cent and quartan 6·8 per cent (Rutherford, 1923). The work was continued till the end of 1924 and the findings of Carter for the period July 1921 to 1924 were:—

*Spleen examinations.*

Number of children examined	..	56,372
Spleen rate per cent	..	13·6

*Blood examinations.*

Number of children examined	..	5,040
Parasite rate per cent	..	13·5

The relative proportions of the different species of malaria parasites were, benign tertian 59 per cent, malignant tertian 10·2 per cent and quartan 32·3 per cent among 1,640 positive out of 10,028 films examined (Thornton, 1925). Carter *et al.* (1927) continued these observations further and their final results were, in the main, similar to the above.

In March 1936 an island-wide spleen census among boys in schools was taken. This was soon after the great malaria epidemic of 1934–35. The rate for the whole island was 30·6 per cent. In 1937 the census was repeated, and a spleen rate of 28·4 per cent was recorded. In 1938 and 1939 combined spleen and parasite surveys were carried out and the results are detailed in this paper.

## SPLEEN AND PARASITE SURVEYS IN 1938 AND 1939.

### SPLEEN SURVEY, 1938.

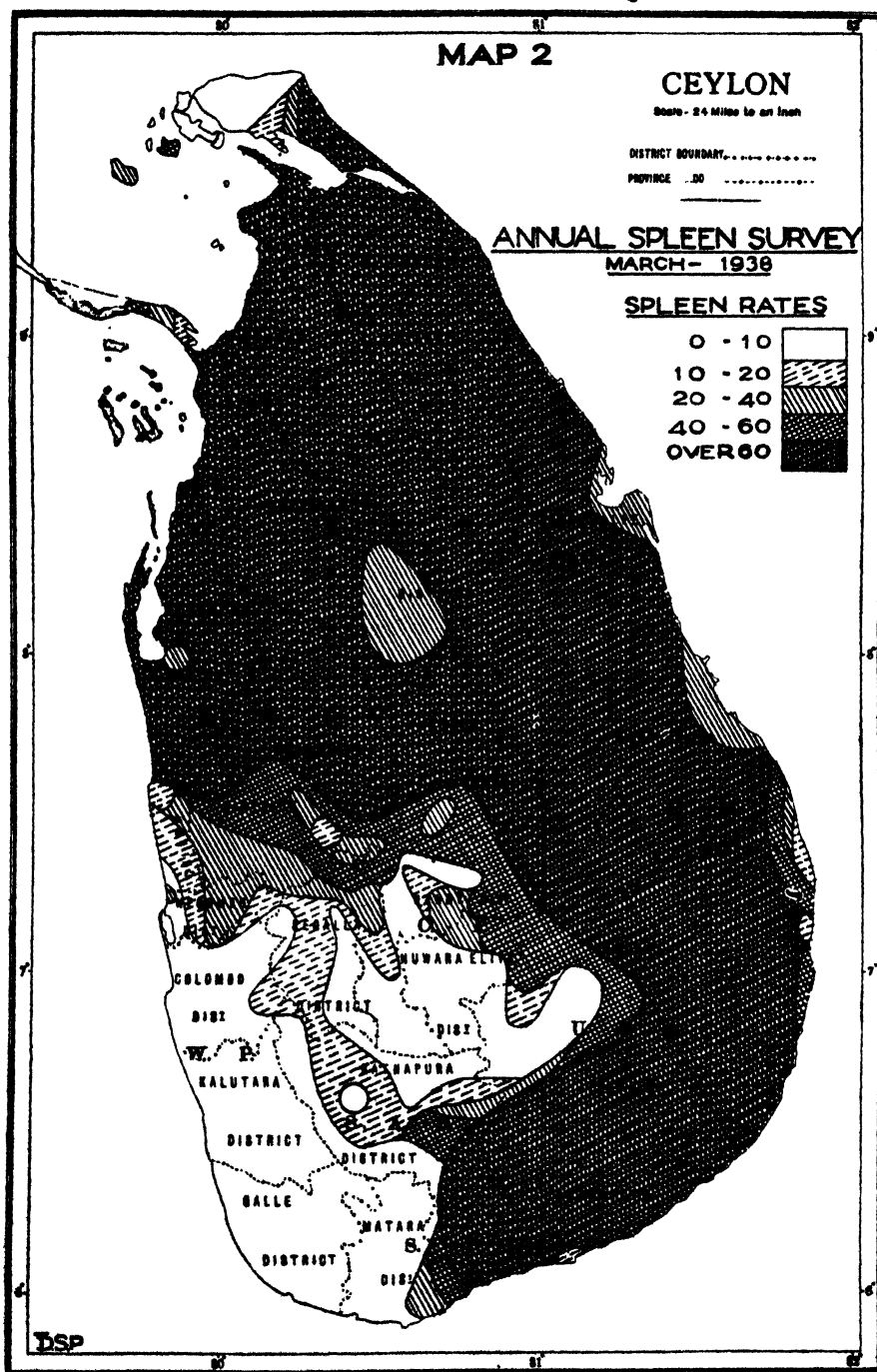
In this survey 144,873 boys in schools were examined during the period February 20 to March 31, 1938 (Map 2).

*The Dry Zone.*—This zone comprises the whole of the Northern, North-Central and Eastern Provinces and the major portions of the North-Western and Uva and smaller portions of Central, Southern and Sabaragamuwa Provinces. The greater portion of the zone is hyperendemic, spleen rates exceeding 60 per cent with the following exceptions:—

(1) The Jaffna peninsula in the north of the island, where rates of less than 10 per cent were recorded over the western half of the peninsula, rising to 10 to 20 per cent and 20 to 40 per cent before merging into the main hyperendemic area.

(2) The eastern littoral extending from the neighbourhood of Trincomalee to the region south of Pottuvil, where spleen rates varied between 20 and 40 per cent in the north portions, while in the south the rates gradually increased from 10 per cent at the coast to 60 per cent inland.







(3) The Island of Mannar showed four degrees of malaria endemicity, with spleen rates of 0 to 10 per cent, 10 to 20 per cent, 20 to 40 per cent and 40 to 60 per cent, increasing progressively from east to west.

(4) An oval area immediately south-east of the town of Anuradhapura with a spleen rate of 20 to 40 per cent, was surrounded by a great mass of hyperendemic areas with rates of over 60 per cent.

(5) The spleen rate in the town of Puttalam was 35 per cent.

(6) The montane regions of Uva of over 3,000 feet, lying in the arc formed by Haputale-Namunukula-Madulsima-Badulla-Hali-ela and Welimada were healthy, with spleen rate of 10 per cent or less. Bordering this area were strips of land of 1,000 to 2,000 feet altitude with rates of 10 to 20 per cent. As the altitude became lower, the spleen rates increased through the 20 to 40 per cent and 40 to 60 per cent ranges, finally becoming merged into the areas with rates of over 60 per cent.

*The Intermediate Zone.*—This zone showed great variations in endemic malaria. The zone comprises the flat lands of the North-Western Province, the montane and submontane areas surrounding Kandy and Matale with altitudes 1,000 to 3,000 feet, the central mountainous regions of parts of Central and Uva Provinces with altitudes 5,000 feet and over, the foot-hills of Sabaragamuwa (1,000 feet and below), and the lowlands of Southern Province. This zone is also plentifully supplied with rivers.

The horizontal portion commencing from the western sea coast passed through areas with spleen rates of 0 to 10 per cent, 10 to 20 per cent, 20 to 40 per cent, and 40 to 60 per cent, until it reached Kurunegala Town, where the rate was 10.9 per cent. This town lay surrounded by an area with spleen rates of 20 to 40 per cent. Along the northern boundary of this zone was a belt of country with a spleen rate of over 60 per cent. The tract of land round Matale and Rattota was of slightly lower endemicity (spleen rates 20 to 40 per cent). In the region of Kandy spleen rates were of the order of 10 to 20 per cent, while in the Galaha-Deltota region, to the east of this, although the altitude is approximately 3,000 feet, the rates were from 20 to 40 per cent, whilst in strips of country to the north and west of Kandy rates of 10 per cent or less were recorded.

The vertical portion commenced in the mountainous belts in the south-east angle of the Central Province and the adjoining portion of Uva located around Pattipola and Ohiya with spleen rates of 0 to 10 per cent, and passed through the province of Sabaragamuwa with increasing rates of 10 to 20 per cent, 20 to 40 per cent, 40 to 60 per cent and over 60 per cent. The southern coastal areas in this zone showed a similar characteristic gradual rise in malaria endemicity, spleen rates of 0 to 10 per cent being recorded in the region of Matara Town and increasing to 20 to 40 per cent, 40 to 60 per cent and over 60 per cent at Hatamune, Beliatta and Weeraketiya respectively.

*The Wet Zone.*—(1) This zone for the most part was very healthy, the spleen rates being for the most part considerably less than 10 per cent. This healthy area comprised the whole of the Western Province except a narrow area in the north, one-third of the Southern Province covered by the sector Bentota-Galle-Weligama-Elpitiya-Deniyaya, the greater portion of the lower third of the Central Province which is the chief mountainous part of the island, varying in altitude between 1,000 feet and 6,000 feet, and two belts of land in the



Province of Sabaragamuwa running along its whole length and contiguous to the adjoining parts of the Western and Central Provinces.

(2) In the narrow strip of land along the northern boundary of the zone, spleen rates of 10 to 20 per cent in the region of Mirigama and 20 to 40 per cent between Polgahawela and Kegalle and at Mawanella and Aranayake were recorded. This strip projected in a finger-like manner into the healthy zone in Central Province mentioned above, as far as Nawalapitiya, with spleen rates of 10 to 20 per cent.

(3) There was a stretch of land in Sabaragamuwa Province running along its whole length, and comprising about one-third of its area, with spleen rates of 10 to 20 per cent.

#### PARASITE SURVEY, 1938.

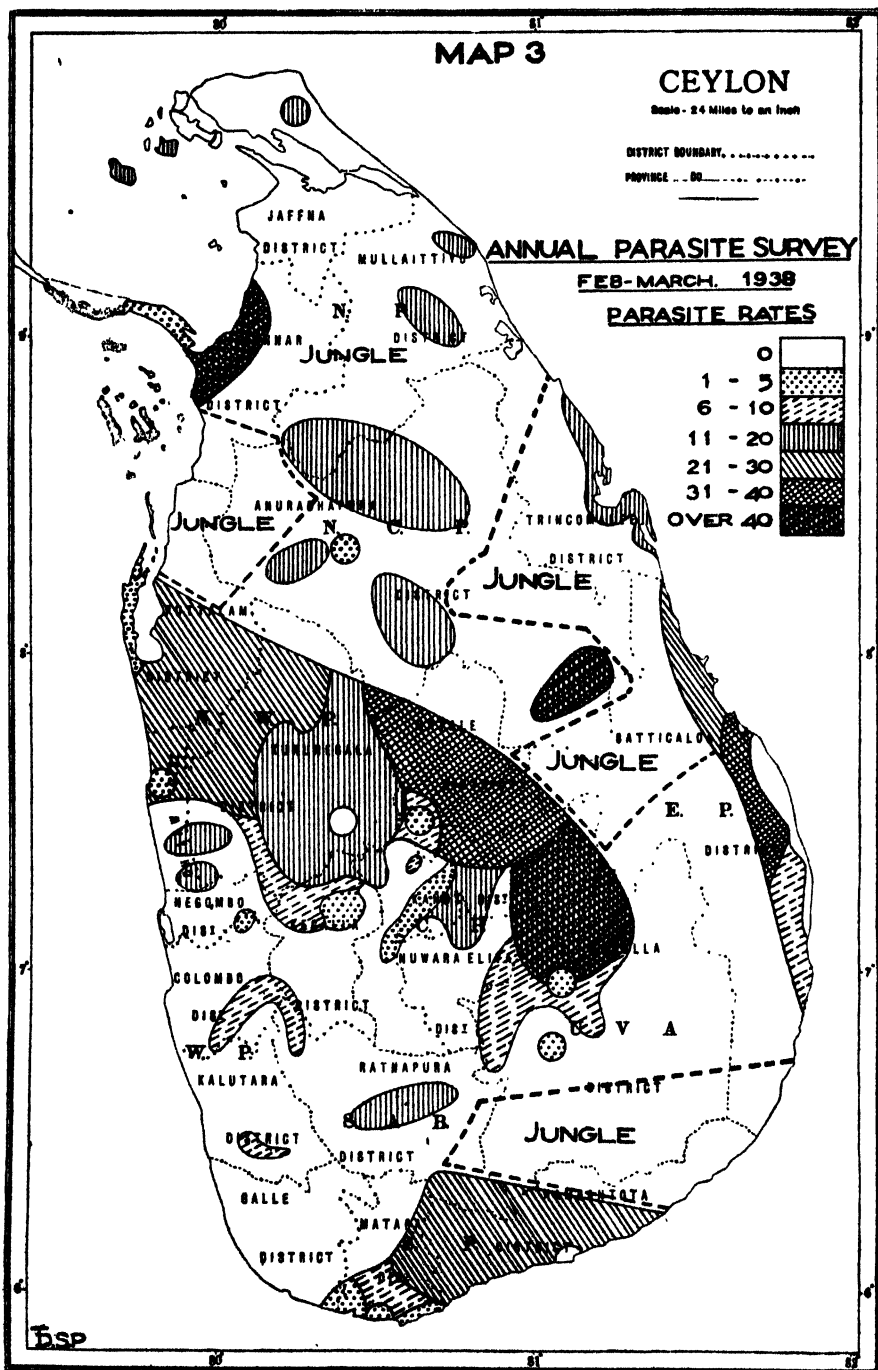
Blood films from 10 per cent of the boys included in the spleen survey were secured for this purpose. The total number of films so examined was 14,653 (Map 3).

*The Dry Zone.*—The parasite rates in this vast area showed great variations. Commencing in the north of the island, nil rates were recorded in the Jaffna Peninsula, except round Kodikamam where the rates were from 11 to 20 per cent. There was a large oval area with rates of 11 to 20 per cent around Madawachchi, while there were two smaller areas, with similar rates to the west of Anuradhapura and centred around Maradankadawala. Parasite rates in Mannar Island were from 1 to 5 per cent, while in the town of Mannar the rate was nil. There was a patch of high parasite rates (over 40 per cent) on the mainland adjoining Mannar. Rates were also high in the broad belt of land lying contiguous to the horizontal part of the Intermediate Zone. The rates in the sector Bingiriya-Maho-Galgamuwa-Puttalam were 21 to 30 per cent, in the Wariyapola area 11 to 20 per cent, and in the Nalanda area 31 to 40 per cent. In the Bintenna regions of Alutnuwara and Bibile the rates were over 40 per cent. The rates in the Passara area were 6 to 10 per cent and in Teldeniya regions 11 to 20 per cent. In the town of Badulla a parasite rate of 1·2 per cent was recorded. The rates in that part of the Southern Province which lies in this zone were from 21 to 30 per cent, with the exception of Hambantota town where the rate was nil.

On the eastern coast, rates of 11 to 20 per cent were recorded in Trincomalee and its neighbourhood. To the north and south of Kalkudah, the rates ranged from 21 to 30 per cent. The rate was nil in the strip of land along the coast commencing just north of Batticaloa and extending to the south of Kalmunai, while in the narrow belt just inland from this strip the rates were 11 to 20 per cent and 6 to 10 per cent.

*The Intermediate Zone.*—From the western coast up to Giriulla, the parasite rate was nil except for an oval area of 11 to 20 per cent extending from Marawila to Dandagamuwa. This was succeeded by a narrow strip with a rate of 6 to 10 per cent and after this by a wide area constituted principally by the south-east quadrant of the North-Western Province with a rate of 11 to 20 per cent. The parasite rate in the town of Kurunegala situated in the centre of this area was nil. The town of Matale yielded a rate of 3·5 per cent and was surrounded by a small area of 6 to 10 per cent, the latter merging into a more extensive area with rates of 31 to 40 per cent. Kandy Town yielded a rate of 1·2 per cent and was surrounded by an area with zero rates, while extending eastwards were small







areas with rates of 1 to 5 per cent and 11 to 20 per cent respectively. Teldeniya lies in the latter area. The figures for Uda Pussellawa-Welimada-Haldummulla area were from 6 to 10 per cent with nil rates in the country north and south of this.

In the Southern Province, rates of 1 to 5 per cent were recorded in the town of Matara and in a narrow belt along the coast, while those in the rest of the area in this zone were chiefly between 21 and 30 per cent.

*The Wet Zone.*—This zone yielded a nil parasite rate with the following exceptions :—

(1) A small area around Veyangoda, with rates of 1 to 5 per cent.

(2) The northern part of Sabaragamuwa Province with rates of 6 to 10 per cent in the Warakapola area and 1 to 5 per cent in the area around Kegalle Town.

(3) The Gampola-Nawalapitiya area in the Central Province (rate 1 to 5 per cent).

(4) A narrow arc of land formed by Homagama-Pugoda-Avisawella-Eheliyagoda (rate 6 to 10 per cent).

(5) A small pocket in the neighbourhood south of Matugama (rate 6 to 10 per cent).

(6) The Pelmadulla-Kahawatta-Balangoda area (rate 11 to 20 per cent).

(7) A narrow belt in Southern Province, lying along the zonal boundary line, with a parasite rate of 21 to 30 per cent inland, and 6 to 10 per cent and 1 to 5 per cent along the sea coast. Weligama and Ahangama are in the last section.

#### PARASITE SPECIES PREVALENCE, 1938.

For each parasite rate area defined in Map 3, the relative percentage distribution of the species of parasites found was determined. The results obtained are shown graphically in Map 4. The percentage values are represented by sizes of sectors of circles, similar to the method adopted by Knowles *et al.* (1930) for a like purpose.

All three species of malaria parasites were found in the island in varying proportions. The quartan parasite was far the most predominant type, and the malignant tertian second, followed closely by benign tertian.

The zonal distribution of the parasites was thus :—

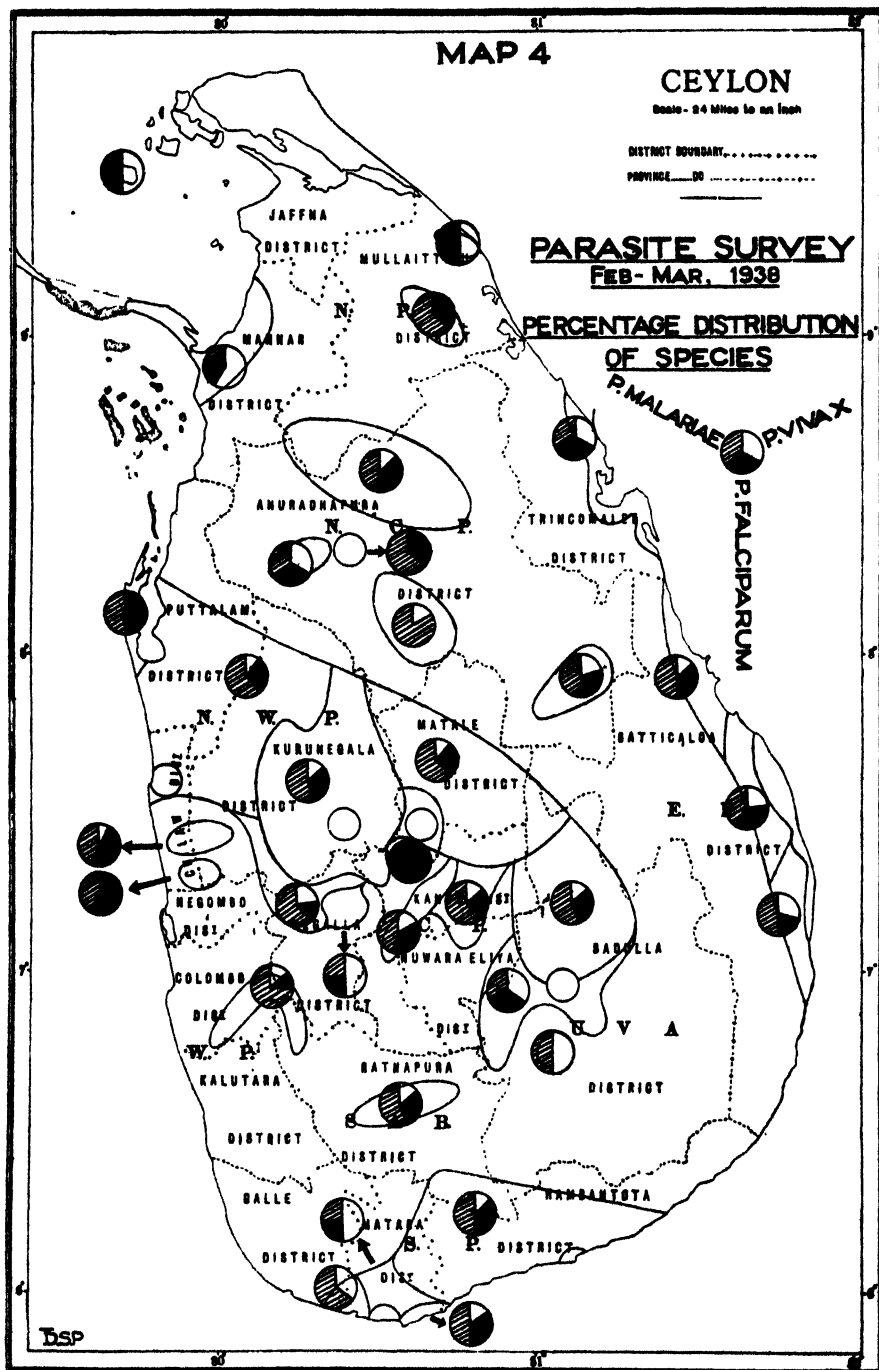
##### *The Dry Zone.*

Number of films examined	..	..	4,516
Number positive	..	..	348
Quartan	..	..	48 per cent
Malignant tertian	..	..	31 per cent
Benign tertian	..	..	21 per cent

##### *The Intermediate Zone.*

Number of films examined	..	..	4,873
Number positive	..	..	268
Quartan	..	..	49 per cent
Malignant tertian	..	..	37 per cent
Benign tertian	..	..	14 per cent







*The Wet Zone.*

Number of films examined	..	..	5,264
Number positive	..	..	48
Quartan	..	..	44 per cent
Malignant tertian	..	..	29 per cent
Benign tertian	..	..	27 per cent

The relative position of the parasites for the whole island generally held good for each of the rainfall zones separately. The quartan parasite held the dominant position and the benign tertian was the least prevalent. But there was a difference in the values obtained for the malignant tertian and benign tertian parasites in each of the zones; for, while the former was appreciably more prevalent in the Dry Zone and more markedly present in the Intermediate Zone, the percentages of the two parasites in the Wet Zone were almost equal. The figures for the quartan parasite were approximately equal in each of the zones.

## SPLEEN SURVEY, 1939.

In this survey 148,504 boys in schools were examined during the period February 20 to March 31, 1939. The results are shown in Map 5.

*The Dry Zone.*—The results generally were similar to those in 1938 with the following chief departures :—

(1) In the eastern half of the Jaffna Peninsula there was a general lowering of the spleen rate to the next range.

(2) Along the eastern littoral, rates of 20 to 40 per cent were recorded in Mullaitivu. The rates in Trincomalee Town and its neighbourhood were reduced to 10 to 20 per cent.

(3) There was a reduction in rates to 40 to 60 per cent over an area situated to the south-east of Anuradhapura Town.

(4) There was a diminution in the rates for Puttalam and for an area to the south of it.

(5) There was a lowering in the spleen rate in the tract of land north of Badulla Town.

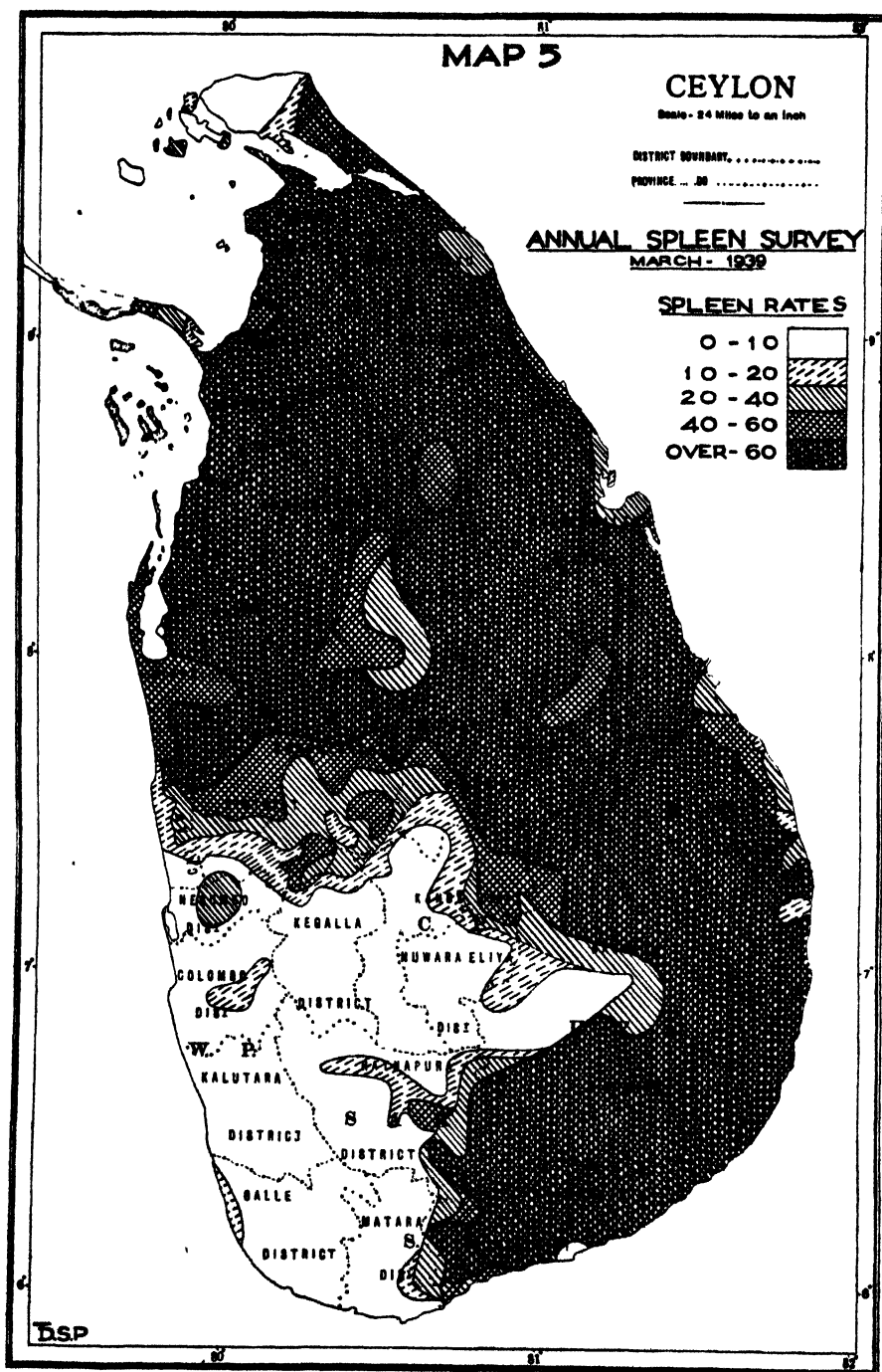
(6) The Wariyapola area and extensive regions around it showed considerable reduction of the spleen rates to the lower ranges of 20 to 40 per cent and 40 to 60 per cent.

*The Intermediate Zone.*—Endemicity in this zone differed markedly from that recorded in 1938. The areas in the horizontal limb, where spleen rates of 40 to 60 per cent and over were recorded in 1938, now yielded rates of 20 to 40 per cent; and rates of 20 to 40 per cent had now reduced to 10 to 20 per cent. The rate in Kurunegala Town was 11.2 per cent, and in Kandy-Matale sector it was less than 10 per cent. The Southern Province areas in this zone showed an extension of malaria endemicity westwards towards the Wet Zone.

*The Wet Zone.*—The survey of this year showed that the areas where spleen rates of 10 to 20 per cent were recorded in 1938 had become contracted, and the figures for the whole zone were 10 per cent or less with the following exceptions :—

(1) Rates were from 10 to 20 per cent in the Kaduwela-Pugoda region.







(2) Rates of 10 to 20 per cent were recorded in a narrow tract along the sea coast extending from Bentota to a point a little to the south of Ambalangoda.

#### PARASITE SURVEY, 1939.

Blood films from 33 per cent of the boys examined for splenic enlargement were examined from the whole of the North-Central, Uva and the Eastern Provinces, the Northern Province except the western half of the Jaffna Peninsula, and the Matale District of Central Province. From the rest of the island, films were obtained from 10 per cent of the boys. A total of 21,690 blood films were examined (Map 6).

#### PARASITE RATES, 1939.

*The Dry Zone.*—The rates in Jaffna Peninsula resembled in the main those recorded in 1938, except for a decrease in the rate round Kodikamam and a new area with 31 to 40 per cent rate along the coast. The areas surrounding Madawachehi and Anuradhapura had become extended but the rates were similar. There was a reduction in the rates in the Bingiriya-Galgamuwa-Puttalam area, and those of Wariyapola and Maho had increased to the next stage. In the remaining areas of this zone there was a general reduction to the next lower scale.

*The Intermediate Zone.*—Parasite rates varied widely in the horizontal limb. There was an extension of the higher rate areas (11 to 20 per cent and over) westwards, while rates in Kurunegala Town and the surrounding region had become reduced from 11 to 20 to nil. There was a general reduction in the parasite rates in the regions to the east of Matale Town, while at Teldeniya the figures ranged from 6 to 10 per cent. In the Southern Province, Matara Town and the coastal strip yielded a nil parasite rate, and there was a lowering of the rates to the next level in the other parts, viz., 11 to 20 per cent.

*The Wet Zone.*—The results were similar to those in 1938 with the following differences :—

(1) A new area with parasite rates of 6 to 10 per cent appeared around Gampaha.

(2) The rate in Kegalle Town was nil.

(3) The rates in Nawalapitiya area were from 6 to 10 per cent.

(4) There was a new area with parasite rate of 6 to 10 per cent running across the middle of Sabaragamuwa Province comprising the Ruwanwella-Eheliyagoda-Balangoda sector.

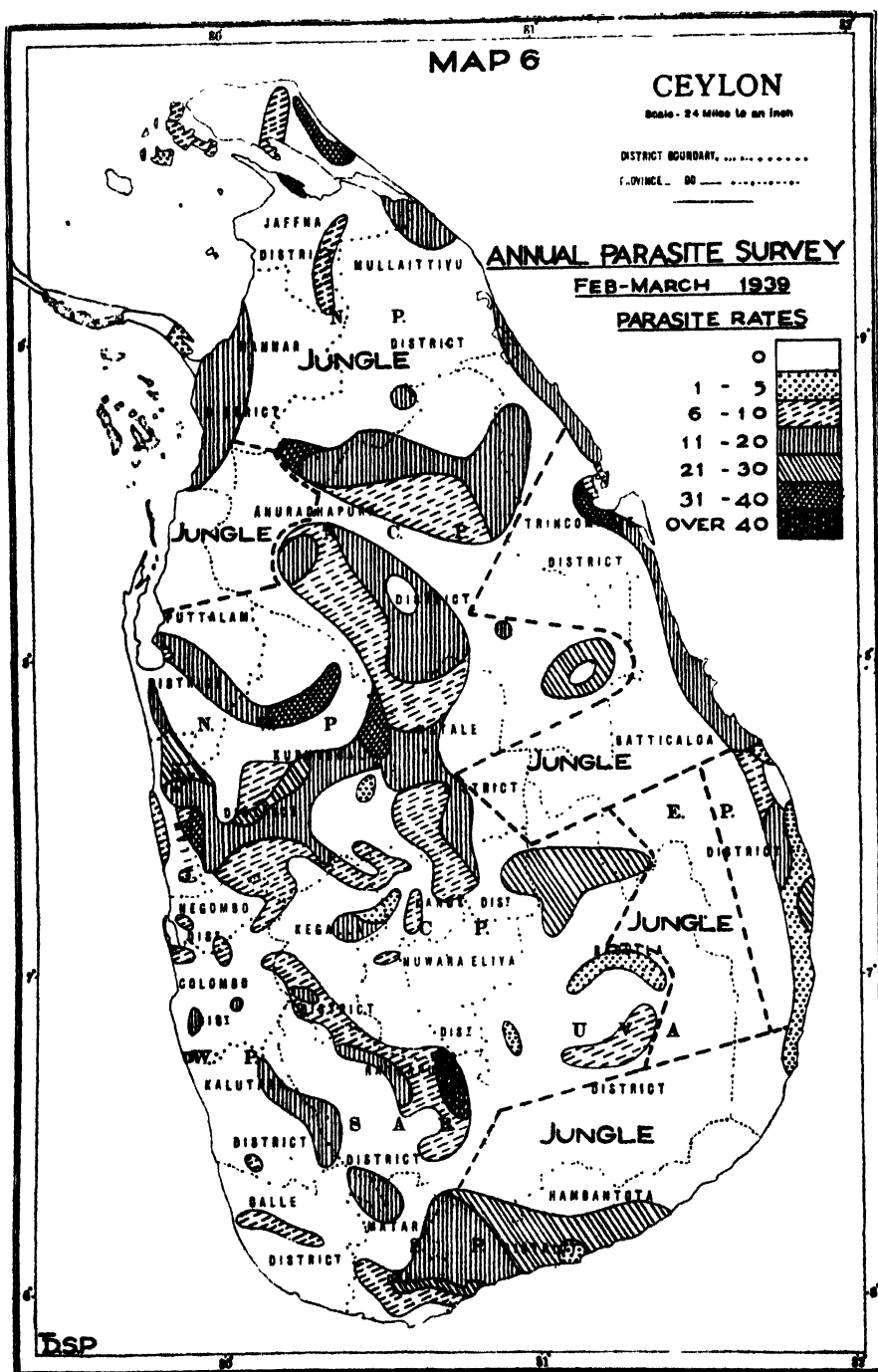
(5) A new finger-like area stretching from Ambalangoda to Baddegama with a rate of 6 to 10 per cent was revealed in the survey.

(6) There was an area including Morawaka and Deniyaya where the rates were from 11 to 20 per cent.

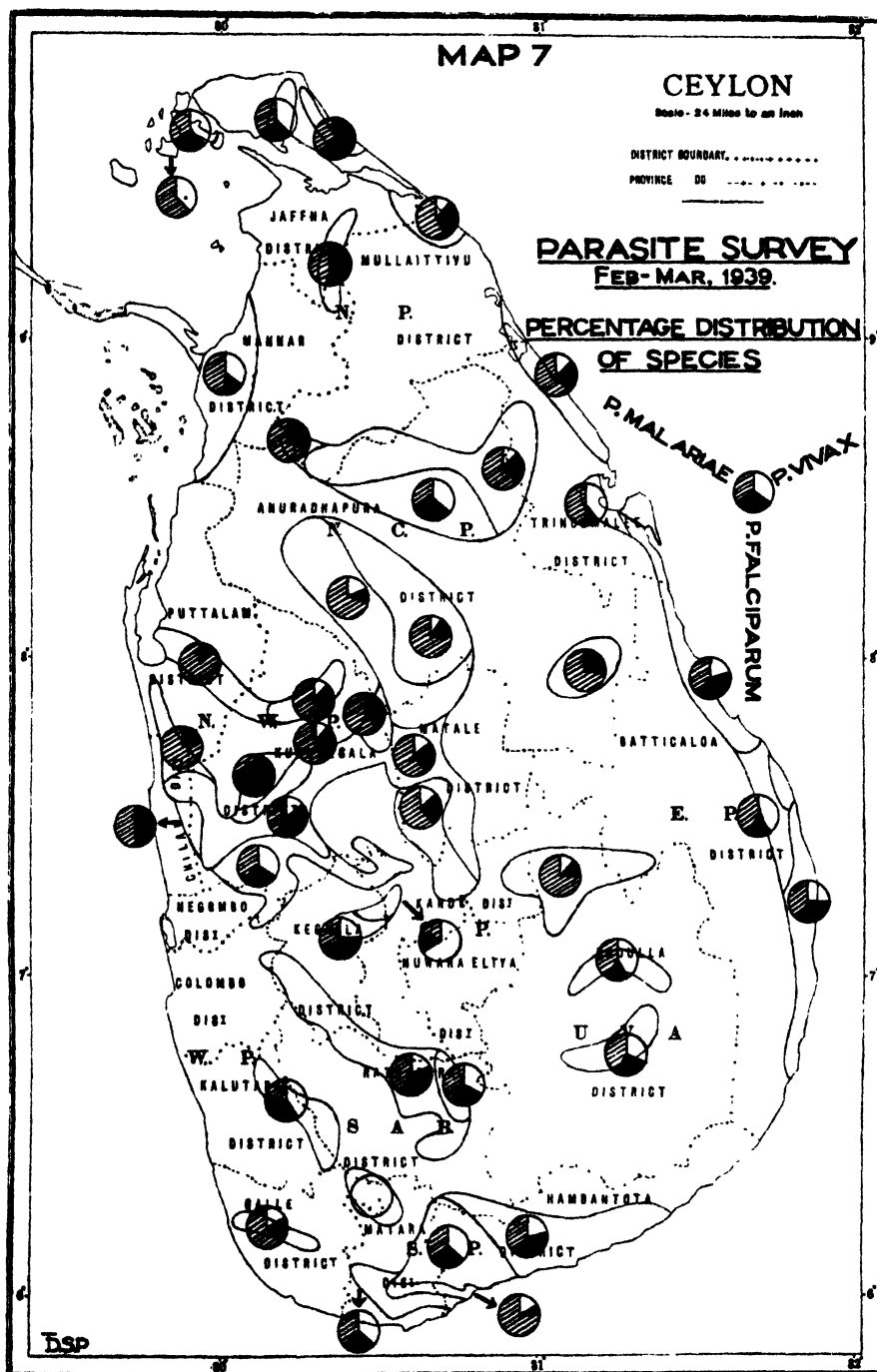
#### PARASITE SPECIES PREVALENCE, 1939.

The results obtained are shown in Map 7. The quartan parasite was seen to be the dominant species in a very large number of localities; the benign tertian and malignant tertian species predominated in a few places only, and appeared almost with equal frequency.











The zonal distribution of the species prevalence was thus :—

*The Dry Zone.*

Number of films examined	..	..	9,765
Number positive	..	..	698
Quartan	..	..	55 per cent
Malignant tertian	..	..	26 per cent
Benign tertian	..	..	19 per cent

*The Intermediate Zone.*

Number of films examined	..	..	5,955
Number positive	..	..	295
Quartan	..	..	52 per cent
Malignant tertian	..	..	29 per cent
Benign tertian	..	..	19 per cent

*The Wet Zone.*

Number of films examined	..	..	5,970
Number positive	..	..	118
Quartan	..	..	30 per cent
Malignant tertian	..	..	33 per cent
Benign tertian	..	..	37 per cent

In their zonal distribution, the quartan parasite was the most predominant in the Dry and Intermediate Zones. In the Wet Zone the benign tertian parasite occupied the first place and quartan was the least prevalent, but the numerical differences between the three species were small. The malignant tertian parasite was intermediate in position in all zones and occurred in approximately equal proportions. The figures for the benign tertian parasite were low in the Dry and Intermediate Zones.

## EPIDEMIOLOGY OF MALARIA IN CEYLON.

Eighteen species of anophelines have been found in Ceylon, and of these only one, viz., *Anopheles culicifacies*, has been incriminated as a malaria vector of importance. Malaria transmission takes place throughout the year in the Dry and Intermediate Zones. In all zones there is a marked accession of the fever during certain months of the year, of such a regularity, that such periods have become to be popularly called 'fever seasons'. The Dry Zone has one fever season yearly during the period November to January, while the other two zones have two seasons, which occur during the months of April to June and November to January. The Dry Zone receives its main rainfall during the north-east monsoon only, and the widespread water collections due to the rains afford breeding places for *A. culicifacies*, with a consequent rise in malaria incidence. The Intermediate and Wet Zones receive rainfall during both monsoons. A deficiency in rainfall produces conditions of drought and the drying up of the many rivers in these areas, with a consequent extensive pool formation in the beds. *A. culicifacies* breeds profusely in these pools, and this may occur at two periods of the year. Thus malaria in the Dry Zone is due to excessive rainfall and in the other two zones to deficiency in rainfall. In the hill-country above 3,000 feet altitude, the vector decreases markedly in prevalence, and malaria is absent or occurs only sporadically.



Epidemics occur in cyclical periods at intervals of 5 or 6 years, and it is the Intermediate Zone that is chiefly involved. Such epidemics follow periods of drought caused by the failure of the monsoon rains.

### DISCUSSION.

The several spleen surveys that have been done from time to time show that the hyperendemic areas are situated mainly in the Dry Zone regions of the island. No appreciable fluctuations in spleen rates occur from year to year, in this zone. The greatest fluctuations in spleen rates occur in the Intermediate Zone, and smaller fluctuations occur in the northern and central portions of the Wet Zone depending on the occurrence of epidemic malaria. The Wet Zone areas normally are healthy, with spleen rates of 10 per cent or less and frequently nil, while the Intermediate Zone in a normal year is intermediate in malaria endemicity between the healthy Wet Zone and the hyperendemic Dry Zone. The spleen rates in the Intermediate and Wet Zones are greatest immediately after an epidemic, and these rates progressively diminish and reach their lowest level during the succeeding five or six years, when the next epidemic breaks out; the occurrence of the epidemic sends the rates up again and such cycles continue to be repeated. Thus at Polgahawela in the Intermediate Zone, the spleen rates for each of the years 1936 to 1939 were 91, 60, 46 and 21 per cent respectively.

A consideration of the many surveys done in the island leads to the view that the spleen rates recorded in the 1939 survey, and shown in Map 5, are the lowest that would be registered in Ceylon at any time. When the endemicity reached this degree, the health condition of the island, from malaria point of view, is at its optimum, and an epidemic outbreak of the disease is now due in accordance with the malaria epidemic cycle natural for the island. With the onset of this outbreak of malaria, the spleen rates are again advanced to a higher level in the area affected, as was revealed by the 1940 survey, which is reserved for a future report.

The diversity in malaria endemicity in this small island is noteworthy. It is, therefore, necessary that different localized areas should be studied separately.

The parasite rates vary a great deal in different regions. Maps 3 and 6 show extensive areas that are unshaded, signifying a zero parasite rate. Proportionately the Dry Zone shows the largest extent of zero rate, next is the Wet Zone, and the Intermediate Zone shows the least. When correlated with the results of the spleen survey (Maps 2 and 5), it is evident that zero parasite rates had been found both in the vast hyperendemic Dry Zone areas with spleen rates of over 60 per cent and in the healthy areas of the Wet Zone with spleen rates of the order of 0 to 10 per cent. A standardized number of microscopical fields of the blood smears were examined in these investigations, and, therefore, the interpretation of the results is different for each of these zones. It is, that in the Dry Zone areas the density of malaria Plasmodia was very low and that the parasites were not numerous enough to be detected by the examination of the microscopical fields adopted as our standard; while in the Wet Zone the zero parasite rate was due to the absence of malaria parasite carriers, as in these regions malaria is only an occasional disease.

The Intermediate Zone shows the greatest variations in parasite rates, and is the area with the largest proportion of parasite carriers. This finding is



important, as this zone is particularly liable to epidemic malaria. The spleen rates are of intermediate values in this zone, and the association of such intermediate spleen rates with high parasite rates is in marked contrast to that in the Dry Zone where high spleen rates are found in association with a zero parasite rate.

In the Wet Zone there are present pockets of high parasite rates but with zero spleen rates. In these localities malaria usually scarcely exists, but on account of the starting of industrial projects, artificial breeding places of *A. culicifacies* are created with a consequent local outbreak. The presence of parasite carriers in such areas is truly due to 'man-made' malaria. Such pockets may increase or decrease according as creation of borrowpits is continued or not.

As regards the different species of malaria parasites, it is seen, from Maps 4 and 7, that the three commoner forms are present throughout the island. The predominance of the quartan parasite during the period February-March is a noteworthy feature of the parasitology of malaria in Ceylon. This is the period when throughout the island seasonal rises have subsided and a condition of low malaria endemicity is beginning to be established. Our findings as to the predominant position of the quartan parasite are in accord with those of Gunasekara (*loc. cit.*), James and Gunasekara (*loc. cit.*) and James (*loc. cit.*) for isolated areas. In the Wet Zone, in 1939, the quartan was not predominant.

The position of *P. falciparum*, as recorded by our work, is different from that found by the previous observers. Their work showed that the malignant tertian parasite was comparatively rare (10 to 15 per cent). But in our surveys in 1938 and 1939 rates of 30-40 per cent were recorded. Our results are based on much larger samples and are representative of the position of species prevalence during the present periods. We are unable to decide whether or not this rise in the prevalence of the malignant tertian parasite has been due to a progressive increase of this species on account of the epidemic outbreak in 1928-29 and the great malaria epidemic of 1934-35. Our surveys show the fluctuation in numbers of the parasite; in the Dry and Intermediate Zones there is a diminution and in the Wet Zone a slight increase. The benign tertian parasite was the least prevalent in all zones except in the Wet Zone in 1939, when it was the most prevalent species.

#### SUMMARY.

1. The Island of Ceylon is divisible into three climatic zones, based on the rainfall during the south-west monsoon period. These are the Dry, Intermediate and Wet Zones according as the rainfall is less than 20 inches, between 20 and 40 inches, and over 40 inches.

2. The degree of malarial endemicity is correlated with these rainfall zones. The Dry Zone is chiefly hyperendemic, the Wet Zone is healthy and the Intermediate Zone is of intermediate endemicity.

3. In the distribution of parasite rates, the Intermediate Zone shows the greatest variation. The zero parasite rate recorded in certain hyperendemic areas is due to the numerical paucity of the parasites, which are therefore not detectable in the standard number of microscopic fields of films examined; that recorded in the Wet Zone is due to the absence of parasites, as malaria is absent or of low endemicity.



4. The three commoner forms of malaria parasites are found everywhere in the Island, *P. malaria* predominating during the period of February-March.

#### ACKNOWLEDGMENTS.

We are thankful to Dr. S. F. Chellappah, O.B.E., Director of Medical and Sanitary Services, for permission to publish this paper, and to our laboratory assistants Messrs. A. K. Piyasena, S. Ambalavanar, T. D. S. Perera and D. C. Goonatilaka for assisting in the examination of the blood films and the drawing of the maps.

#### REFERENCES.

- BAHR, P. H. (1913) .. .. Report on malaria in Kurunegala. Sessional paper 32 *Government Printer*, Colombo.
- CARTER, H. F., ASSISTED BY RUSTOMJEE, K. J., and SARAVANAMUTTU, E. T. (1927). Report on malaria and anopheline mosquitoes in Ceylon Sessional paper 7. *Ibid.*, Colombo.
- CHRISTOPHERS, S. R., and KHAZAN CHAND (1924). Measurement in centimetres of the enlarged spleen in children and its correlation for size of child by a factor based on an anthropometric measurement. *Ind. J. Med. Res.*, **11**, p. 1065
- DEMPSTER, T. E. (1848) .. Notes on the application of the test of organic disease of the spleen as an easy and certain method of detecting malarious localities in hot climates. Reprinted in *Rec. Mal. Surv. Ind.*, **1**, 2, p. 69.
- GUNASEKARA, S. T. (1913) .. Report on the antimalarial campaign at Kurunegala Sessional paper 37. *Government Printer*, Colombo.
- JAMES, S. P. (1914) .. .. Report of sanitary inquiries Jaffna and Northern Ports. Sessional paper 4. *Ibid.*, Colombo.
- JAMES, S. P., and GUNASEKARA, S. T. (1913). Report on malaria at the Port of Talaimannar. Sessional paper 34. *Ibid.*, Colombo.
- KNOWLES, R., and SENIOR WHITE, R., ASSISTED BY DAS GUPTA, B. M. (1930). Studies in the parasitology of malaria. *Ind. Med. Res. Mem.*, **18**, p. 4.
- PERRY, A. (1909) .. .. Ceylon Administration Reports for 1908. Medical. *Government Printer*, Colombo.
- Idem* (1910) .. .. *Ibid.* for 1909.
- Idem* (1914) .. .. *Ibid.* for 1912-13.
- Idem* (1915) .. .. *Ibid.* for 1914.
- RUTHERFORD, G. J. (1916) .. .. *Ibid.* for 1915.
- Idem* (1917) .. .. *Ibid.* for 1916.
- Idem* (1919) .. .. *Ibid.* for 1918.
- Idem* (1923) .. .. *Ibid.* for 1922.
- SCHÜFFNER, W. A. P. (1919) .. Two subjects relating to the epidemiology of malaria. *Meded. Burgerlijk. Geneesk. Dienst. Nederl.-Indië*. Part 9, p. 1. Translated and reprinted in *J. Mal. Inst. Ind.*, **1**, p. 221 (1938).
- SINTON, J. A. (1925) .. .. Notes on the thick film method of examination for malarial parasites. *Ind. J. Med. Res.*, **12**, p. 537
- THORNTON, G. (1925) .. .. Ceylon Administration Reports for 1924. Medical. *Government Printer*, Colombo.







THE NATURAL HOST OF *PLASMODIUM GALLINACEUM*  
(BRUMPT, 1935).

BY

COLONEL H. E. SHORTT, M.D., CH.B., D.S.C., D.T.M. & H., I.M.S.,  
K. P. MENON, L.M. & S., L.R.C.P. & S.,

AND

P. V. SEETHARAMA IYER, M.A.  
(*King Institute of Preventive Medicine, Guindy, Madras.*)

[June 23, 1941.]

IN 1935, Brumpt described a *Plasmodium* found in the domestic fowl in certain countries of Asia, to which he gave the name *Plasmodium gallinaceum*. In the same note, he records the few occasions on which a *Plasmodium*, presumably of this species, has been found in the domestic fowl. The parasite is not a common one, as is evident from the large number of negative examinations carried out on these birds, but we are informed by Mr. M. Crawford of the Department of Agriculture, Peradeniya, Ceylon, in a personal communication, that it is not infrequently found among domestic fowls in Ceylon.

The question naturally arises as to the source of the infection in these birds. The transmitting mosquitoes are various species of *Aedes*, all of which are very efficient vectors. Those of which we ourselves have definite knowledge as efficient vectors are *A. aegypti*, *A. albopictus* and *A. vittatus*. It is evident, therefore, that, given the parasite in its natural host in proximity to domestic fowls, there will be nothing to prevent the latter, extremely susceptible as they are, becoming infected.

The rarity of the infection in domestic stocks, however, would appear to indicate either that the infection is rare, even in the natural host, or that the latter does not often come into close contact with domestic stocks.

It appeared to us that some significance might be attached to the fact that in all the places where the infection has been recorded in domestic fowls, the wild jungle fowl, original progenitor of the domestic stocks, was to be found in the adjacent forests.



A search of the literature at our disposal discloses only two records of pigmented parasites in the wild jungle fowl. Plimmer (1914) records a species of *Hæmoproteus* in the jungle fowl in Malaya, and Scott (1926) records the occurrence of a species of either *Plasmodium* or *Hæmoproteus* in *Gallus varius*, the jungle fowl of West Africa. It was decided, therefore, to examine as many specimens of the South Indian jungle fowl (*Gallus sonneratii*) as could be obtained for the presence of *Plasmodium* parasites.

It soon became evident that such parasites could not be common, at least in the area around Madras, because the examination of some forty birds yielded only one carrying a *Plasmodium*. This parasite has been further studied with a view to determining whether it is identical with *P. gallinaceum*.

### COMPARISON OF MORPHOLOGY.

A detailed morphological study reveals no difference between this parasite and the strain of *P. gallinaceum* maintained in this laboratory, which was supplied by Mr. M. Crawford. The number of merozoites in segmenting forms varies between 8 and 30, as in the case of *P. gallinaceum*. There was also no difference in the exo-erythrocytic forms in the brain and organs.

### CROSS IMMUNITY TESTS.

In these tests, two series of birds were prepared, one infected with the Ceylon strain, which came from the domestic fowl, and the other with the Madras strain from the jungle fowl. The birds of each series were about a month old when infected. The infection in these birds was allowed to become chronic, the birds being treated with small doses of quinine when necessary to prevent deaths in the acute stages of infection. The infection was considered to be a chronic one after a lapse of about two months. At this stage, parasites were usually absent from the peripheral blood on microscopical examination, but, in most cases, the persistence of a low-grade infection could be demonstrated by inoculation of the blood into susceptible young chicks. Such birds were considered to have developed an active immunity against the homologous parasites.

The next stage in these tests was the inoculation of the birds of each series with the heterologous strain of parasite, i.e., the Madras strain of parasite into birds immunized to the Ceylon strain and vice versa. The results of the cross immunity tests are given in the table.

As will be apparent from the table, the results appear unequivocally to demonstrate that immunity acquired against the Ceylon strain is fully potent against superinfection with the Madras strain and vice versa. This would appear to indicate the probable antigenic identity of the two strains. In the table, the details of two experiments only (Fowls 41 C and 1 M) have been included in order to show that the results, when the superinfecting dose is with the homologous strain, are the same as when it is with the heterologous strain.

In one instance only, it will be seen, the hyperinfecting dose was followed by the appearance of parasites in the peripheral blood. These, however, were extremely few in number and there was no tendency for them to increase; so it is surmised that they are most likely to represent the original infection, in this case, with the Madras strain.



TABLE.  
*Cross immunity experiments.*

Serial number of fowls.	Date of original infection.	Strain of the parasite.	Infection by blood or sporozoites ?	Date of super-infection	Strum of the parasite.	Injection of blood or sporozoites ?	Time interval between the first and second infection (days).	Result of superinfection.
<b>A.</b>								
37 C	21.i.41	Ceylon	Blood	7.iv.41	Madras	Blood	76	Negative
41 C	8.ii.41	"	"	7.iv.41	"	Sporozoites	58	"
03 C	8.ii.41	"	"	7.iii.41	"	Blood	27	"
56 C	26.ii.41	"	"	19.iv.41	"	Sporozoites	52	"
1 M	27.ii.41	Madras	"	5.iv.41	Ceylon	"	37	"
2 M	6.iii.41	"	"	7.iv.41	"	Blood	32	"
3 M	6.iii.41	"	"	14.iv.41	"	"	39	"
8 M	15.iii.41	"	Sporozoites	18.iv.41	"	Sporozoites	34	"
11 M	19.iii.41	"	Blood	19.iv.41	"	"	31	* Negative
<b>B.</b>								
41 C	8.ii.41	Ceylon	Blood	19.iii.41	Ceylon	Sporozoites	39	Negative
1 M	27.ii.41	Madras	"	20.iii.41	Madras	"	21	"

\* *Note.*—11 M showed two parasites in the blood smear after about 5 minutes' search on 5.v.41. Subsequent examinations showed no increase in the intensity of infection even after 5 days, showing that the infection was probably the residual infection from the blood inoculation on 19.iii.41.



## CROSS AGGLUTINATION TESTS.

Mulligan, Russell and Mohan (1940) have described the agglutination of sporozoites of *P. gallinaceum* by immune serum from fowls carrying a chronic infection with this parasite. The authors have been able to confirm these findings, although none of the sera used by them gave titres as high as those described by Mulligan *et al.* We were able, however, to make use of this reaction as another confirmatory test pointing towards the identity of our parasite with *P. gallinaceum*.

Birds with chronic infection, as in the cross immunity tests, were used as sources of immune serum. Mosquitoes containing sporozoites of the Ceylon strain of *P. gallinaceum* were dissected in varying dilutions of immune serum from birds of both the Ceylon and Madras strains. There was no difference in behaviour with the sera of these two strains, both producing well-marked agglutination of the sporozoites. When the experiment was repeated using sporozoites of the Madras strain, the same results were obtained. These tests, therefore, furnish further evidence as to the identity of these two strains.

## SUMMARY.

1. The original host of *P. gallinaceum* (Brumpt, 1935) is probably the wild jungle fowl, the species varying with locality.

2. Morphology, cross immunity and cross agglutination tests point to the identity of the strains in wild and domestic fowls.

## REFERENCES.

- BRUMPT, E. (1935) .. .. *C. R. Acad. Sci.*, **200**, p. 783  
MULLIGAN, H. W., RUSSELL, P. F., *J. Mal. Inst. Ind.*, **3**, 4. p. 573.  
and MOHAN, B. N. (1940).  
PLIMMER, H. G. (1914) .. .. *Proc. Zool. Soc. Lond.*, p. 181.  
SCOTT, H. H. (1926) .. .. *Ibid.*, p. 231.



## THE STAGE OF *PLASMODIUM GALLINACEUM* FOUND IN THE INCUBATION PERIOD—SECOND OBSERVATION.

BY

P. V. SEETHARAMA IYER, M.A.,  
COLONEL H. E. SHORTT, M.D., CH.B., D.SC., D.T.M. & H., I.M.S.,

AND

K. P. MENON, L.M. & S., L.R.C.P. & S.  
(*King Institute of Preventive Medicine, Guindy, Madras.*)

[June 23, 1941.]

SHORTT, MENON and IYER (1940) have recorded the finding of exo-erythrocytic forms of *P. gallinaceum* on the sixth day of the incubation period after the inoculation of sporozoites into a young chick.

This observation has since been repeated on two occasions, and we consider it to be reasonably established that this form of the parasite is that characteristic of the incubation period and the form from which the blood parasites are presumably derived.

The present note is written to record the finding of similar forms on the fifth day of the incubation period.

The details of these observations are briefly given in the table.

We believe the only reason that these forms have not previously been seen is that they are present in very small numbers and require very careful search for their disclosure. They are in all respects typical in appearance of the forms found in abundance at a later stage of the infection. We were at first under the impression that a massive dose of parasites was more likely to reveal 'incubation-period' forms in the brain than smaller doses, but we are not sure now that this is necessarily the case. As will be seen from the table, in none of the 'positive' cases were sporozoites from more than five *Aedes aegypti* inoculated and, in the case in which incubation-period forms were found on the fifth day, sporozoites from only one *A. aegypti* were used. It is still possible, however, that the greater the dose, the more likely are incubation-period stages to be seen, if by greater dose we mean a larger proportion of fully mature and infective sporozoites. It is quite possible that the sporozoites from



TABLE.

*Details of the finding of incubation-period stages of P. gallinaceum in the brain of chicks.*

Chick number.	Number of <i>A. ægypti</i> used as source of sporozoites.	Date and method of inoculation.	Date of brain examination.	Result.	REMARKS.
24	2	7.iii.1940 Intramuscular.	13.iii.1940	Positive	6 days
43	5	4.iv.1940	10.iv.1940	Positive	6 days
23	1	7.iii.1940	14.iii.1940	Negative	7 days
98	15	30.vii.1940	5.viii.1940	Negative	6 days
132	12	2.x.1940	8.x.1940	Negative	6 days
137	Brain material on sixth day of incubation period used in place of sporozoites.	8.x.1940 Intraperitoneal.	14.x.1940	Positive	6 days
9	1	4.i.1941	9.i.1941	Positive	5 days
145	23	18.x.1940	23.x.1940	Negative	5 days
36	3	27.i.1941	31.i.1941	Negative	4 days
38	2	30.i.1941	3.ii.1941	Negative	4 days
57	7	27.ii.1941	3.iii.1941	Negative	4 days

one mosquito might have more infective mature forms than those from many mosquitoes at a less favourable stage, as it has more than once been suggested that the sporozoites may not be infective until they have spent a certain time in the salivary glands.

We believe that only intensive search is needed to reveal the presence of still earlier stages of the parasite during the incubation period of the infection.

#### SUMMARY.

The finding of incubation-period forms of *Plasmodium gallinaceum* on the fifth day of the incubation period is recorded.

#### REFERENCE.

- SHORTT, H. E., MENON, K. P., and *Ind. J. Med. Res.*, **23**, p. 273.  
 IYER, P. V. S. (1940).



## MALARIA CONTROL BY SPRAY-KILLING ADULT MOSQUITOES\*.

### THIRD SEASON'S RESULTS.

BY

PAUL F. RUSSELL,

AND

FRED W. KNIPE.

[July 15, 1941.]

### INTRODUCTION.

THIS paper reports observations during a third season of experiments with spray-killing adult anophelines to control malaria in South Indian villages. Two previous papers have been published (Russell and Knipe, 1939; 1940). Our results during the third season, discussed below, amplify and fully confirm the observations of the first two seasons.

### SPRAYING TECHNIQUE.

The equipment used during our third season's work was of two types: (a) hand-pumped air tanks and (b) power-filled air tanks. These sources of air operated a well-known household paint spray-gun.

The first type consisted of ordinary spray pressure tanks, equipped with hand-operated air pumps. None was found exactly adapted to local use; therefore, they were modified to meet requirements. All tanks were equipped with pressure-reducing regulators, which insured a constant pressure on the spray-gun nozzle at all times. This regulator is essential for efficient operation, whether air pressure is obtained from hand-operated or from power-operated air pumps.

Operation of the hand-pumped tank proceeded as follows:—The tank was pumped up by the operator, usually to a pressure of about 50 lb. per square inch. The operator then slung the tank on his back, taking the spray-gun in

\* These studies were a part of the programme of Malaria Investigations, a project under the auspices, and with the support, of the International Health Division of the Rockefeller Foundation, co-operating with the Pasteur Institute of Southern India, Coonoor, and the Madras Provincial Health Department.



his hand, and proceeded with the spraying operations. These operations continued until the air pressure in the tank dropped below 15 lb. per square inch. One tank of air of about 50 lb. pressure was usually sufficient to last for from two to three minutes, and, in the hands of an efficient operator, was enough to spray one small house. Larger houses required two or three pumpings, depending upon their size.

When a tank of air became exhausted, the operator removed it from his back and again pumped up pressure. This pumping operation is a weak point in the system. Pumping time is *wasted time* in a spray-killing programme. Furthermore, it is hard work, especially with light-weight, underfed labour. The labourers actually spent more time pumping and resting than spraying.

In view of this inherent drawback in using hand-pumped tanks, a second method of securing air pressure was tried out, namely using power-filled tanks.

In this method, a petrol-driven air compressor, equipped with a suitable reservoir and mounted on hard rubber wheels, was used as the source of compressed air. This outfit maintained a constant pressure of 90 lb. per square inch in the reservoir.

Individual tanks of sufficient strength to withstand 90 lb. pressure were strapped to the backs of labourers. These tanks, equipped with pressure regulators, were filled from the main air reservoir in exactly the same way as an automobile tyre is filled at a service station. The labourer then proceeded with spraying in the same manner as with the hand-operated unit. This method is to be recommended over the hand method because of its ease of operation.

There are disadvantages which may offset the great advantage of ease of operation. First costs are high and a personnel must be employed that understand the operation of a petrol-driven machine. Another disadvantage is that the unit must be transported—not always an easy task in a South Indian village.

Each type has its place in a malaria control programme, where spray-killing of adult mosquitoes is the technique used. Both methods have been successfully used in the local programme. Other and better types of equipment are in process of development.

Four different units have been tried out in experiments, each having distinctive features rendering it adaptable to certain conditions. These units were as follows :—

- (1) Solidified carbon dioxide (dry ice) used for generating spraying pressure;
- (2) Hand-pumped air in pressure tanks;
- (3) Power-pumped air in pressure tanks; and
- (4) Improvements in the ordinary hand-operated 'flit gun'.

Solidified carbon dioxide equipment was reported upon by Knipe (1941). No improvement in equipment or technique was developed in 1940. This method of application is considered the best and most efficient thus far developed. But its use in India is practically prohibited because of the high cost of solidified carbon dioxide.

The type of outfit most suitable for spray-killing must be cheap, simple in operation, sturdy, and light in weight. Hand-operated pressure tanks now come nearest meeting such conditions. Various outfits that approach these



requirements have been tested during the past year. All were modified to some extent. The conclusions drawn were that many hand-operated outfits may be considered satisfactory for the purpose, but in order to ensure maximum efficiency, certain individual modifications are necessary. For instance, all tanks should be equipped with an automatic air pressure-reducing regulator. Air pressure-reducing regulators ensure a uniform flow of air at a definite pressure through the spray nozzle. This conserves air and spraying material. By conserving air less pumping is necessary, and thus less labour is required. We adopted a type of well-known commercial hand-operated pressure tank, which we equipped with a special pressure-reducing regulator. The outfit was light in weight, and very easy to operate. It was simple in construction and the air pump was sturdy. The only criticism was that the tank seams were not very well made and frequently broke open. A tank built of slightly heavier material with welded seams would serve the purpose much better.

There are communities in which malaria control may be attempted by spray-killing, and where a petrol power-driven air compressor could be used advantageously. An attempt has been made to develop equipment for such communities. Air can be compressed for this unit by any small, portable compressor driven by a petrol engine. The outfit should be mounted on four rubber wheels. The spraying unit itself was even more simple than the hand-operated apparatus, consisting only of a pressure tank carried on the labourer's back.

This tank should be built to withstand higher pressures than the hand-pumped outfit, since the air supply is delivered to it at relatively high pressure. One outfit used in our experiments could withstand 90 lb. per square inch pressure and had a safety factor of three.

This tank was equipped with an automobile valve through which air was introduced. It was equipped with the same type of air reduction valve as the hand-operated type. It was carried on the labourer's back and did not need to be detached for refilling with air. Refilling was done by another labourer who connected the tank to the main reservoir of the petrol-driven outfit in exactly the same way as an automobile tyre would be connected for inflation at a service station.

Speedy operation is the advantage of this type of tank, in that continuous filling of several tanks may be conducted at one time. There is no delay and no tiring of the coolie by hand pumping.

The ordinary hand-operated sprayer ('flit gun' type) is neither substantial in construction, nor economical in consumption of insecticide. It may be satisfactory for individual houses where it is used only occasionally and where some waste of insecticide is of no economic importance. But in extensive adult spray-killing, a more substantial outfit which does not waste insecticide is desirable.

An effort has been made to determine the weaknesses of existing models in order to improve upon them. Some of the weaknesses frequently found were: -

1. Use of too light-weight material;
2. Poor construction, especially at seams and joints;
3. Plungers and plunger leathers inadequate;
4. Poorly constructed and adjusted vaporizing units; and
5. Hand grip needed on plunger barrel.



The first three of these weaknesses could be easily rectified in a properly constructed gun. In fact, improvements have been made by several manufacturers.

Poorly constructed vaporizing units seem to prevail on practically all models we have tried out. Not one unit has given as good vaporization as the cheapest type of paint spray-gun, when operated in the ordinary way. On the other hand, when air pressures are increased to 20 lb. or more per square inch, every outfit tested gave good results. But it is quite impossible for a labourer to maintain 20 lb. pressure on the nozzles of existing outfits.

We believe we now know most of the weaknesses of these hand-operated units, and will make an effort during the present year to overcome most or all of them. We also believe we can design and build an outfit, capable of withstanding hard usage, which will develop a well-vaporized spray. This outfit must be low in initial cost and easy to operate.

We carried out spraying experiments in four villages, the technique differing somewhat in each village. In each case, we sprayed only in the daytime, starting as early in the morning as possible.

#### KASANGADU.

Kasangadu is the original village in which spray-killing of adult mosquitoes was tried and about which our earlier reports were written (Russell and Knipe, 1939; 1940). This was the third year of operation in the village. The only variation in technique from former years was a change of equipment. Hand-operated pressure tanks were used in place of the 'flit guns' employed in former years. As in previous years, pyroicide 20 was the insecticide used, being diluted with kerosene at the rate of 19 parts of kerosene to one part of pyroicide 20. The time required to spray the village remained the same, i.e., 2½ days. The interval between spraying operations remained the same, i.e., 7 days.

The number of buildings sprayed remained approximately the same. A few new houses were erected, but this added work was easily taken care of within the 2½ days' time limit.

TABLE I.  
*Statistics of villages sprayed in 1940.*

Village.	Number of houses.	Number of out-houses and cow-sheds.	Total number of buildings.	Population of village.
Kasangadu ..	358	191	549	1,790
Attikkottai ..	183	74	257	915
Perumalkovil ..	149	28	177	745
Modalcheri ..	66	16	82	330

Costs for the season are shown in Table I. It should be noted that *per capita* costs have been markedly reduced over the 1939 costs. This reduction



can be accounted for by the more efficient equipment which was used. This *per capita* cost is approaching the theoretical limit set as being within the possibilities of local funds which might be available for malaria control.

#### ATTIKKOTTAI.

Attikkottai is a village in which, for a second season, equipment has been given a thorough trial before being used in other villages. The buildings in Attikkottai were sprayed once weekly as in Kasangadu, with pyroicide 20. But the equipment varied as development of that equipment proceeded.

A careful record of costs was maintained in so far as possible, but the cost *per capita* per season in Attikkottai was somewhat high, because of the varying methods of application. Also, structures in Attikkottai were larger than those in the other experimental village.

#### PERUMALKOVIL.

This village was first sprayed in 1940, and an attempt was made to reduce *per capita* costs by introducing two new factors. Unfortunately, it was impossible to secure the services of a competent supervisor for the operations, which fact, it is believed, influenced both the cost figures and the effectiveness of control.

In order to reduce cost, the interval between spray applications was increased to nine days during the early weeks of the transmission season. After October, the interval was still further increased to ten days. This resulted in a reduction in spray applications from 22 (uniform season as explained below) for a seven-day interval in Kasangadu, to 16 spray applications in Perumalkovil. The result, as compared to Kasangadu, was a reduction in *per capita* cost from Re. 0-5-8 to Re. 0-4-8, as shown in Table VI. Had supervision been efficient, it is believed this reduction would have been greater.

Whether or not the increased interval between sprayings adversely affected malaria control cannot be stated for certain. Table IX indicates that some degree of malaria control was effected. Another year of trials will be required before this point can be cleared up.

#### MODALCHERI.

Modalcheri was sprayed for the first time in 1940. The technique used was exactly the same as in Kasangadu, spray-killing being done at seven-day intervals. Identical equipment and personnel were used, under the same supervisor as in Kasangadu.

The only difference between Modalcheri and Kasangadu was in material used. In Modalcheri, a home-made extraction obtained from Pyrethrum flowers, grown in the Nilgiris District of Madras Province, was used.

Here again, an attempt was made to lower the *per capita* cost. Local Pyrethrum flowers were purchased for Re. 1 per lb., and from one pound of such flowers approximately two gallons of insecticide were made at a cost of Re. 1-4-7 per gallon (pre-war price) as compared to pyroicide 20 cost of Rs. 2-3-5 per gallon (pre-war price). This is a reduction in cost of 41-8 per cent in insecticide alone.

The Indian-grown Pyrethrum extract seemed to be as effective an insecticide in laboratory tests as is pyroicide 20.



The method of extracting the locally-grown *Pyrethrum* flowers was as follows: One imperial gallon (160 oz.) of kerosene was used as the extracting agent. One pound of chopped flowers was soaked in one-half gallon of kerosene in an air-tight container for 48 hours with occasional shaking. The resultant liquor was decanted and stored in an air-tight dark coloured bottle. The flowers were then soaked in one-quarter gallon of kerosene for 24 hours, with occasional shaking, and the liquor decanted and stored. The flowers were then placed in a long narrow funnel and the remaining quarter gallon of kerosene was allowed to drip slowly, drop by drop, through the flowers. The remaining flowers' residue was then pressed in a home-made wooden press, the liquor collected, and all four liquors mixed together, making approximately 145 oz. of extract from one pound of flowers.

The relative potency of the four extractions has not yet been tested. For the present it is sufficient to state that the resulting combined extract, when mixed with an equal part of kerosene (1 to 1) apparently had as good killing qualities as spraying mixture prepared from commercial pyroicide 20. The cost was about half the cost of the standardized mixture (1 part pyroicide 20 to 19 parts kerosene), when *Pyrethrum* flowers cost Re. 1 per lb. and kerosene Re. 0-13-1 per gallon.

#### SPRAYING COSTS.

In order to compute total spraying costs for 1940, the form shown in Table II was used. Explanations follow:—

*Total cost of mixture (1940)* is the actual cost of materials used under prices existing in 1940. These were higher than in former years.

*Labour and supervision costs* were calculated on a uniform basis for all villages.

*Rent* was charged for equipment storage in each village.

*Incidentals* were charged against each village as they occurred.

Using total costs for each village for 1940 as summarized in Table II, and the statistics of villages (Table I), certain interesting statistics are derived, as shown in Table III. The most important of these are 1940 costs *per capita* per season. These figures are for the actual spraying season of 1940; the total number of rounds sprayed varying with each village, because spraying in individual villages was continued until the catch of *A. culicifacies* dropped below 5 per man-hour.

In order to reduce relative figures to a uniform basis for estimating comparable *per capita* costs, a uniform season of 22 weeks was chosen. This season theoretically extends from July 1 to December 1. The estimated cost per uniform season is shown in Table IV. In this table the apparent effect of a reduction in estimated number of rounds per uniform season in Perumalkovil becomes apparent in *per capita* cost per season results.

Table V represents total spraying costs in 1940 when these are computed at pre-war rates. For instance, concentrated pyroicide 20 cost Rs. 31-0-0 per gallon at pre-war rates, and now costs Rs. 45-0-0 per gallon. The relative costs of kerosene were: pre-war Re. 0-11-2, and current Re. 0-13-1 per gallon. These higher prices materially affect total costs for the year.

Comparative estimated costs per uniform season of 22 weeks based on 1939 and 1940 data (pre-war rates) are shown in Table VI. Based on these data



TABLE II.  
*Total spraying costs, 1940.*

Village.	Material used.	Total mixture used.		Cost per gallon.		Total cost of mixture.		Labour and supervision.		Depreciation on equipment.		Rent.		Incidentals.		Total cost.	
		Gals.	Oz.	Rs.		Rs.		Rs.		Rs.		Rs.		Rs.		Rs.	
Kasangadu	Pyrocide 20 mixture.	195	120	3-0-5		592-5-7		131-4-0		25-14-0		6-0-0		6-0-6		761-8-1	
Attikkottai	Pyrocide 20 mixture.	137	100	3-0-5		416-7-4		65-11-4		13-2-0		6-0-0		1-4-0		502-8-8	
Perumalkovil	Pyrocide 20 mixture.	80	120	3-0-5		244-5-9		48-12-0		8-14-0		6-0-0		3-1-0		311-0-9	
Modalcheri	Indian-grown pyrethrum 1 : 1 mixture.	43	46	1-6-8		65-13-3		23-8-0		3-8-0		6-0-0		4-12-0		103-9-3	

*Notes.*—160 oz. = 1 Imperial gallon.  
 1 Rupee = £0-1-6 or \$0-32 approximately (1940).  
 Cost of pyrocide 20 .. Rs. 45-0-0 per gallon (1940).  
 " " Indian-grown Pyrethrum Re. 1-0-0 " pound (1940).  
 " " kerosene .. " 0-13-1 " gallon (1940).  
 The amount of spray mixture used in Kasangadu in 1940 was approximately one ounce per 2,350 cubic feet sprayed.



TABLE III.  
*Spraying statistics—summary.*

Village.	Total rounds sprayed.	Number of coolies.	Spray hours per round.	Total structures.	Average number of structures per man*.	Total mixture used.	Average number of structures sprayed per gallon†.	Total cost.	Cost per structure per season.	Cost per structure per round.	1940 cost per capita per season.
						Gals. Oz.		Rs.	Rs.	Re.	Re.
Kasangadu ..	21	5	20	549	5.49	195 120	58.8	761-8-1	1-6-2	0-1-1	0-6-9
Attikkottai ..	19	7	6	257	6.12	137 100	38.1	502-8-8	1-15-3	0-1-8	0-8-9
Perumalkovil ..	18	5	5	177	7.08	80 120	39.6	311-0-9	1-12-1	0-1-7	0-6-9
Modalcheri ..	24	3	4	82	6.83	43 46	45.6	103-9-3	1-4-3	0-0-10	0-5-0

\* Size of house, distance between houses, and total number of animal shelters in different villages account for the wide variation in these figures.

† Size of house and number of animal shelters in different villages affect these figures.



TABLE IV.

*Estimated cost per uniform season (based on actual costs).*

Village.	Total rounds sprayed.	Actual season cost <i>per capita</i> .	Estimated rounds per uniform season (July 1 to Dec. 1).	Estimated cost <i>per capita</i> per uniform season.
		Rs.		Rs.
Kasangadu ..	21	0-6-9 (1940).	22 (weekly)	0-7-2
Attikkottai ..	19	0-8-7 (1940).	22 (weekly)	0-10-2
Perumalkovil ..	18	0-6-9 (1940).	16 (ten-day interval).	0-5-11
Modalecheri ..	24	0-5-0 (1940).	22 (weekly)	0-4-7

it appears that *per capita* costs in Kasangadu have been considerably lowered during the 1940 season. Thus, it becomes evident that some factor has markedly influenced cost. Since equipment was the only change made in Kasangadu, it appears that efficient equipment must be given this credit.

The results in Attikkottai, in 1939 are not comparable with those for 1940 and are omitted. No spraying was done in Modalecheri and in Perumalkovil during 1939.

Comparison of 1940 estimated *per capita* costs, for all villages, with costs in Kasangadu for 1939, indicates that very considerable progress has been made in bringing such *per capita* costs within an economically practical range for application in South Indian villages.

#### EFFECT ON ANOPHELINES.

As in previous years, some effect on the density of adult *A. culicifacies* (the vector) was noted. In Table VII are shown data with reference to collections of adult *A. culicifacies* per man-hour in the sprayed village of Kasangadu and the contrast village of Tuvarangurichchi, for the years 1938 to 1940. Collections were made a day or two before spraying. The peak collection in Kasangadu in 1940 was 16.1 in June. This is to be contrasted with an August peak of 24.8 in Tuvarangurichchi. In Tuvarangurichchi there was an uninterrupted rise from 0.3 in April to 24.8 in August. In Kasangadu the rise from 0.0 in March to 16.1 in June was succeeded by a decline to 2.0 in October. There was a rise in November to 2.8 and in December to 4.0. Spraying was stopped on November 10.

As in previous years, in 1940 a decrease in larval density was not very clear. Density of *A. culicifacies* larvæ, per collection positive for this species, rose from 0.0 in April to 32.9 in July, thereafter declining (Table VIII). In Tuvarangurichchi, densities increased unevenly from March to August, thereafter declining. Possibly the earlier decline in Kasangadu may have been a result of



TABLE V.  
Total spraying costs, 1940, computed at pre-war rates.

Village.	Material used.	Total mixture used.	Cost per gallon.	Total cost of mixture.	Labour and supervision.	Depreciation on equipment.	Rent.	Incidentals.	Total cost.
		Gals.	Oz.	Rs.	Rs.	Rs.	Rs.	Rs.	Rs.
Kasangadu ..	Pyrocidc 20 mixture.	195	120	2-3-5	433-5-3	131-4-0	25-14-0	6-0-0	602-7-9
Attikkottai ..	Pyrocidc 20 mixture.	137	100	2-3-5	304-10-3	65-11-4	13-2-0	1-4-0	390-11-7
Perumalkovil ..	Pyrocidc 20 mixture.	80	120	2-3-5	178-12-0	48-12-0	8-14-0	3-1-0	245-7-0
Modalcheri ..	Indian-grown pyrethrum 1:1 mixture.	43	46	1-4-7	58-14-7	23-8-0	3-8-0	4-12-0	96-10-7

See notes for Table II.

Cost of pyrocidc 20 ..	Rs. 31-0-0 per gallon (pre-war price).
" " Indian-grown pyrethrum	Re. 1-0-0 " pound ( " " ).
" " kerosene	" 0-11-2 " gallon ( " " ).



spray-killing. But it would appear that the primary effect of the spraying is to kill infected *A. culicifacies* before they become infective. It does not appear that our results are due to a lowering of the general density of the vector species.

TABLE VI.

*Comparative estimated costs per uniform season (July 1 to December 1) based on 1939 and 1940 data (pre-war rates).*

Village.	Number of rounds per uniform season (July 1 to Dec. 1).	1939 Estimated cost per capita per uniform season.	1940 Estimated cost per capita per uniform season.
		Re.	Re.
Kasangadu ..	22 (weekly)	0-12-0	0-5-8
Attikkottai ..	22 (weekly)	Not comparable	0-7-11
Perumalkovil ..	16 (ten-day interval).	Not sprayed	0-4-8
Modalcheri ..	22 (weekly)	Not sprayed	0-4-4

### EFFECT ON MALARIA.

The effect of spray-killing on malaria is illustrated by Tables IX to XI. Reference to Table IX will show that, while the spleen and parasite rates in the contrast village of Tuvarangurichehi have remained the same or become a little higher, the rates in Kasangadu have fallen notably. Spleen rates in Kasangadu have dropped from 68 per cent in the 1937 malaria season to 6, in 1940. Corresponding parasite rates have gone from 57 per cent to zero. In this village as shown in Table X, spleen size has also been reduced. No spleens larger than size 1 have been found in 1939 or 1940. So, too, as shown in Table XI, the infant parasite index in Kasangadu indicates that we have successfully interrupted the transmission of malaria by our spraying procedure.

Effects in the other villages are not so clear, because, in the case of Attikkottai, we have been more interested in testing new apparatus, and in the case of the other two villages we did not begin spraying until 1940.

However, a downward trend in spleen and parasite indices is apparent in each case. The spleen rate in Attikkottai in 1939 was 36 and this had dropped to 10 per cent in 1940. The parasite rate declined from 11 to 8 per cent. In Modalcheri, the malaria season spleen rates in 1937 and 1938 were 80 and 82 per cent respectively. In 1940, the rate was only 28 per cent. The parasite rates were 65 and 71 per cent in 1937 and 1938 respectively, whilst in 1940 this figure had fallen to 25 per cent. In Perumalkovil, the spleen rates in 1937 and 1938 malaria seasons were 39 and 43 per cent respectively. In 1940, this rate was 32 per cent. The corresponding parasite rates were 31 and 41 per cent in 1937 and 1938, respectively, whilst in 1940 the figure recorded was 28 per cent.



TABLE VII.

*A. culicifacies* adults collected in Kasangadu and Tuvarangurichchi,  
1938 to 1940, by months, by man-hour collections.

Description.	KASANGADU.			TUVARANGURICHCHI (CONTRAST).		
	1938.	1939.	1940.	1938.	1939.	1940.
January ..	11.5	5.1	4.1	8.2	5.9	7.1
February ..	5.9	1.6	3.5	5.3	1.2	5.0
March ..	4.1	1.0	0.0	2.8	2.8	0.8
April ..	3.1	4.8	0.8	2.5	0.9	0.3
May ..	0.7	4.3	0.5	0.2	3.3	1.9
June ..	0.5 *	3.9 *	16.1 *	0.8	3.5	19.3
July ..	6.1	10.4	8.8	8.3	17.6	21.1
August ..	14.7	14.3	8.4	27.9	28.4	24.8
September ..	5.0	4.9	6.1	10.7	17.6	15.5
October ..	4.2	3.1	2.0	7.2	10.6	4.4
November ..	3.0	2.3	2.8	5.5	7.5	6.4
December ..	3.8	3.4	4.0	6.4	4.3	4.8
Average ..	5.2	4.9	4.8	7.2	8.6	9.3

*Notes.*—No control work at all in Tuvarangurichchi.

Spray-killing each week in Kasangadu .. { June 13 to Dec. 31, 1938.  
June 15, 1939 to Jan. 15, 1940.  
June 27 to Nov. 10, 1940.

Irrigation water in canals in both villages .. { June 15, 1938 to Jan. 1939.  
June 15, 1939 to Jan. 1940.  
May 15, 1940 to Jan. 1941.

\* Collections prior to first spraying

## DISCUSSION.

It is of interest to report that the villagers like spray-killing and have given full co-operation to the operating staff. They state that it gives them complete relief from mosquito bites on the night of the day spraying took place, and some relief during the following two nights. They have noticed the diminution in amount of fever. They have not complained of the odour of the spray or of any ill effect on their food or household belongings. They have not objected to the labourers entering all parts of their houses. We have made it a rule in each village to use local lads of good caste for the spraying.



TABLE VIII.

*Anopheline larvæ collected in Kasangadu and Tuvarangurichchi, 1938 to 1940.*

Month.	KASANGADU.				TUVARANGURICHCHI.			
	All anophelines.		<i>A. culicifacies</i> only.		All anophelines.		<i>A. culicifacies</i> only.	
	Total.	Per positive collection.	Total.	Per collection positive for <i>A. culicifacies</i> .	Total.	Per positive collection.	Total.	Per collection positive for <i>A. culicifacies</i> .
Jan. 1938 ..	401	33.4	83	9.2	256	17.1	86	9.6
Feb. ..	1,626	33.2	418	10.4	1,064	19.0	169	5.6
Mar. ..	1,503	37.6	213	9.2	1,255	27.3	197	8.6
Apr. ..	1,161	29.0	93	5.2	867	39.4	106	10.6
May ..	1,407	54.1	100	6.2	389	18.5	7	2.1
June ..	1,049	30.0	72	6.0	1,195	38.5	74	9.2
July ..	2,965	60.5	575	16.4	3,578	73.0	830	23.0
Aug. ..	2,504	59.6	857	23.2	3,445	67.5	1,102	26.2
Sept. ..	3,281	67.0	1,219	29.7	1,332	83.2	310	28.2
Oct. ..	678	21.9	277	10.6	1,528	34.0	451	13.7
Nov. ..	1,193	54.2	362	22.6	771	26.6	120	7.5
Dec. ..	559	32.9	183	20.3	1,266	45.2	171	8.6
Jan. 1939 ..	529	27.8	269	22.4	1,480	64.3	328	27.3
Feb. ..	795	44.2	289	20.6	567	27.0	95	15.8
Mar. ..	771	48.2	44	4.9	293	19.5	13	4.3
Apr. ..	319	31.9	75	25.0	279	34.9	28	7.0
May ..	1,141	67.1	199	19.9	835	41.8	271	27.1
June ..	755	47.2	127	12.7	538	29.9	62	6.9
July ..	281	35.1	46	9.2	693	40.8	102	8.5
Aug. ..	698	36.7	249	17.8	1,608	55.4	402	16.1
Sept. ..	548	28.8	220	14.7	987	47.0	408	20.4
Oct. ..	622	31.1	112	9.3	689	38.3	215	15.4
Nov. ..	394	20.7	100	8.3	707	39.3	128	9.1
Dec. ..	489	32.6	118	13.1	421	16.8	113	8.1



TABLE VIII—concl'd.

Month	KASANGADU.				TUVARANGURICHCHI.			
	All anophelines.		<i>A. culicifacies</i> only.		All anophelines.		<i>A. culicifacies</i> only.	
	Total	Per positive collection.	Total.	Per collection positive for <i>A. culicifacies</i> .	Total.	Per positive collection.	Total.	Per collection positive for <i>A. culicifacies</i> .
Jan. 1940 ..	444	34.2	172	21.5	671	32.0	197	15.2
Feb. ..	592	37.0	180	13.8	313	31.3	19	3.8
Mar. ..	108	27.0	2	2.0	137	19.6	2	1.0
Apr. ..	11	11.0	0	0.0	109	15.6	21	21.0
May ..	229	20.8	16	8.0	204	18.5	22	11.0
June ..	719	44.9	251	20.9	791	39.6	168	12.9
July ..	572	57.2	296	32.9	1,514	68.8	451	28.2
Aug. ..	888	43.6	480	24.0	1,129	59.4	508	29.9
Sept. ..	615	41.0	296	21.1	1,250	65.8	474	26.3
Oct. ..	642	35.7	278	21.4	656	34.5	327	23.4
Nov. ..	428	28.5	190	19.0	598	29.9	244	22.2
Dec. ..	500	33.3	208	18.9	539	27.0	178	14.8

It seems to us that this point of pleasant contact with the people is important, for it might lead to co-operation in more permanent malaria control and to the easier introduction of other public health measures.

Spray-killing must be classed as an ever-recurring measure. There is no evidence that by three years' spraying in Kasangadu we have reduced the potential malariousness of the place as regards the mosquito vector, although we have succeeded in temporarily eliminating gametocyte carriers. One would always prefer to do 'permanent' malaria control, but the cost of such work in Kasangadu is out of the question. The place is surrounded by extensive *A. culicifacies* breeding places during the irrigation season, and it is doubtful if larval control could be effected for less than Rs. 5 *per capita* per year. Permanent protection might be given if one spent on permanent works such as filling, revetting canals, and providing proper drainage, the cost of, say, 10 years of spray-killing. Some larval control would, however, still be required each year after the completion of 'permanent' work. Therefore, spray-killing cannot lightly be dismissed as a temporary measure, any more than the continuous chlorination of drinking water in a city can be frowned on as an ever-recurring



TABLE IX.

*Spleen and parasite rates in sprayed and contrast villages, 1937 to 1940.*

Description.		KASANGADU.		ATTIK-KOTTAI.		MODAL-CHERI.		PERUMALKOVIL.		TUVARAN-GURICHCHI (CONTRAST).	
Spleen rates.	Season.	Number examined.	Percentage with enlarged spleen.	Number examined.	Percentage with enlarged spleen.	Number examined.	Percentage with enlarged spleen.	Number examined.	Percentage with enlarged spleen.	Number examined.	Percentage with enlarged spleen.
1937	Off	50	42	..	..	35	60	49	29	40	43
1938	Off	73	41	..	..	43	47	57	30	46	41
1939	Off	77	9	48	23	..	..	..	..	63	48
1940	Off	88	3	57	21	..	..	..	..	70	51
1937	Malaria	56	68	..	..	20	80	36	39	33	55
1938	Malaria	104	24	..	..	49	82	37	43	100	61
1939	Malaria	97	15	55	26	..	..	..	..	61	57
1940	Malaria	103	6	78	10	67	28	65	32	93	58
Parasite rates.	Season	Number examined.	Percentage with parasites.	Number examined.	Percentage with parasites.	Number examined.	Percentage with parasites.	Number examined.	Percentage with parasites.	Number examined.	Percentage with parasites.
1937	Off	50	38	..	..	10	70	49	20	40	35
1938	Off	73	40	..	..	43	40	57	26	46	37
1939	Off	77	4	48	15	..	..	..	..	63	33
1940	Off	88	0	57	12	..	..	..	..	70	31
1937	Malaria	56	57	..	..	20	65	36	31	33	46
1938	Malaria	104	12	..	..	49	71	37	41	100	52
1939	Malaria	97	6	55	11	..	..	..	..	61	48
1940	Malaria	103	0	78	8	67	25	65	28	93	50

*Notes.*—Kasangadu sprayed 1938, 1939, 1940.

Attikkottai sprayed 1939, 1940.

Modalcheri and Perumalkovil sprayed 1940.

Tuvarangurichchi—contrast village, no control at all.



TABLE X.

*Spleen sizes in Kasangadu and Tuvarangurichchi.*

Description.	Date and season.		Per cent of palpable spleens in each size-group.				
			PI.	1.	2.	3.	4.
Kasangadu (sprayed)	1937	M	36.8	23.7	15.8	13.2	10.5
	1938	O	33.3	30.0	16.7	13.3	6.7
		M	60.0	36.0	4.0	0.0	0.0
	1939	O	71.4	28.6	0.0	0.0	0.0
		M	57.1	42.9	0.0	0.0	0.0
	1940	O	66.7	33.3	0.0	0.0	0.0
		M	50.0	50.0	0.0	0.0	0.0
Tuvarangurichchi (contrast)	1937	M	22.2	22.2	44.4	11.1	0.0
	1938	O	31.6	31.6	26.3	10.5	0.0
		M	26.2	34.4	14.8	16.4	8.2
	1939	O	26.7	33.3	23.3	13.3	3.3
		M	28.6	20.0	22.9	22.9	5.7
	1940	O	36.1	38.9	11.1	11.1	2.8
		M	33.3	31.5	20.4	11.1	3.7

Notes.—O = Off season.

M = Malaria season.

PI = Palpable on deep inspiration.

public health measure. If local free labour could be obtained for this locally popular measure, the annual costs might be small enough to warrant permanent reliance on spray-killing, however much this goes 'against the grain' of the average malariologist. Certainly, we can heartily recommend spray-killing, under South Indian village conditions, as a measure to 'hold the fort' until more permanent measures are possible. We do not believe that a cost of 5 annas *per capita* per year is unreasonable, and we hope to reduce this further. Had free labour been available in Modalcheri, the *per capita* costs would have been Re. 0-3-11 or about U. S. \$0.08\*. This sum is certainly not beyond the financial ability of the local and provincial governments.

\* One Indian rupee consists of 16 annas, each anna having 12 pies. At present rates of exchange Re. 1/- is approximately equivalent to U. S. \$0.32 or £0-1-6.



PLATE XXII.



Fig. 1. Spraying outfit with Hudson pressure tank and DeVilbiss paint gun. The tank on left is as purchased. Note that tank on right, as used, has been modified by installation of regulating valve. The outlet spout from the tank has been changed and put in a new position.

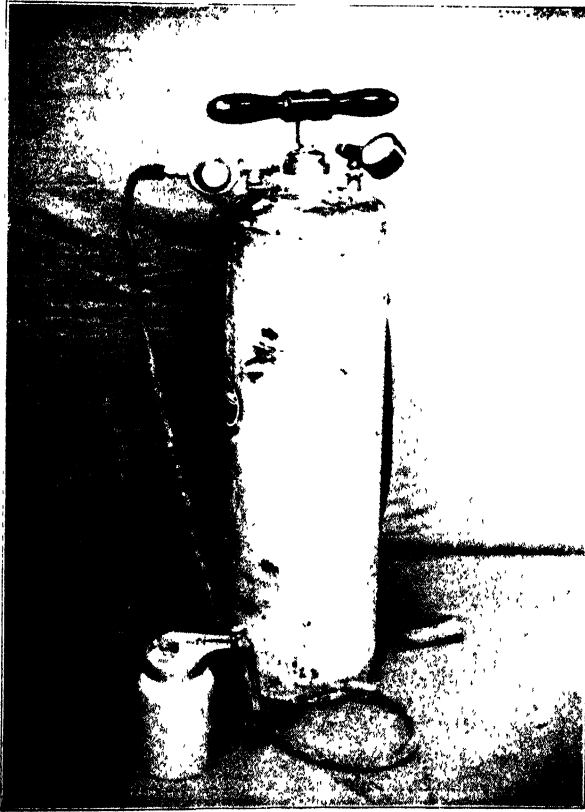


Fig. 2. Spraying outfit with air-oil pressure tank and DeVilbiss paint gun. This tank has been equipped with a regulating valve and also with a pressure gauge.



PLATE XXIII.



Fig. 3 Spraying outfit in operation in the cow-shed portion of a dwelling



Fig. 4. Main street in Modalcheri. Note labourers with spraying equipment.



TABLE XI.

*Infant parasite indices in Kasangadu and Tuvarangurichchi, 1937 to 1941.*

Description.	Date.	Season indexed by infant survey.	NUMBER OF INFANTS.		Per cent positive.
			Examined.	Positive.	
Kasangadu .. ..	Jan.-Feb. 1938.	M. S. 1937	16	2	12.5
	Feb. 1940	M. S. 1939	24	0	0.0
	June 1940	O. S. 1940	22	0	0.0
	Jan. 1941	M. S. 1940	24	0	0.0
Tuvarangurichchi (contrast)	Jan.-Feb. 1938.	M. S. 1937	14	3	21.4
	Feb. 1940	M. S. 1939	22	5	22.7
	June 1940	O. S. 1940	21	3	14.3
	Jan. 1941	M. S. 1940	27	5	18.5

Notes.—M. S. = Malaria season.

O. S. = Off season.

Since the malaria season in this area extends from July 1 to the following February 1, the examination of infants not over a year old in late January and in February is primarily an index of transmission during the immediately preceding June-January malaria season, when adult spray-killing was in progress in Kasangadu in 1938, 1939 and 1940, but not in 1937, and not in any year in Tuvarangurichchi.

### SUMMARY.

An account is given of a third season's experiments in the control of malaria in some South Indian villages by spraying dwellings with pyrethrum insecticides to kill adult anophelines of the vector species in their daytime resting places. The third season fully confirmed and extended the observations of the first two years in showing that the method is effective in greatly reducing malaria transmission. Costs were reduced by better technique, and now approach, if they do not entirely meet, the economic requirements of the area in respect of money potentially available for malaria control.

### REFERENCES.

- KNIFE, F. W. (1941). .. The use of solidified carbon dioxide in developing pressure for spray-killing adult mosquitoes in malaria control. *Amer. J. Trop. Med.* (In press.)
- RUSSELL, P. F., and KNIFE, F. W. (1939). Malaria control by spray-killing adult mosquitoes. First season's results. *J. Mal. Inst. Ind.*, **2**, 3, pp. 229-237.  
Also see correction slip. *Ibid.*, **3**, 1 (1940).
- Idem* (1940) .. Malaria control by spray-killing adult mosquitoes. Second season's results. *Ibid.*, **3**, 4, pp. 531-541.







## MALARIA IMMUNITY IN THE RHESUS MONKEY.

BY

B. B. DIKSHIT.

*(Inquiry under the Indian Research Fund Association.)**(From the Haffkine Institute, Bombay.)*

[July 17, 1941.]

TALIAFERRO and MULLIGAN (1937) have discussed and demonstrated the significance of the lymphoid macrophage system in defence against malaria. Coggeshall and Kumm (1937), on the other hand, have shown that the serum of immune monkeys possesses a protective and to a certain extent curative property against malaria. Experimental evidence is thus available which shows that both the 'cellular' and 'humoral' agencies play a part in malarial immunity. Mulligan and his co-workers (1940) have recently discussed the present position of our knowledge regarding these two agencies, and have presented important experimental evidence which leads them to conclude that immunity in malaria 'involves the interaction of both "cellular" and "humoral" agencies and is probably fully effective when a full measure of one of these factors operates in the presence of an adequate measure of the other'. In the present paper, evidence is presented which attempts to show the rôle the cellular and humoral agencies play individually in malarial immunity.

## MATERIALS AND METHODS.

Rhesus monkeys (*Silenus rhesus*), usually weighing between 3.5 and 4.5 kg., were used in these experiments. The strain of *Plasmodium* used was the K<sub>2</sub> strain of *Plasmodium knowlesi* which was originally obtained from the Malaria Institute of India, and which has been serially passaged in this Institute for the last three years. The 'immune' monkeys used were those in which a primary infection had been induced and treated with atebirin, which had a relapse that was also treated, which had more than one superinfection to increase the antibody concentration in their blood, and which usually harboured a very low degree of infection in their peripheral blood. In some experiments, 'immune' monkeys free from parasites in their peripheral blood were used, and this freedom was achieved by administration of sulphathiazole (Dikshit and Ganapathi, 1940). Sufficient time was allowed in these experiments for the sulphathiazole to disappear completely from the body. For the sake of



convenience, blood obtained from 'immune' monkeys is referred to below in this paper as 'immune blood' and cells of the lymphoid macrophage system as 'immune cells'.

The principal object in these experiments was to determine the rôle which the cells and the blood play in giving protection against malaria. To achieve this end, the blood of 'immune' monkeys was removed as completely as possible and normal blood obtained from normal monkeys transfused into them to restore their original blood volume. The 'immune' animals thus had their own 'immune cells' and normal 'non-immune blood'. Similarly the blood of normal monkeys was removed and substituted with blood obtained from 'immune' monkeys, so that the normal animals had their own 'non-immune cells' and 'immune blood'. Such animals, with their blood replaced, were then infected with fresh parasites to see if they were still susceptible to infection. The removal and replacement of blood were effected by the methods briefly described below. In all such experiments direct matching of the blood was made before replacement was effected.

#### HEPARIN METHOD.

Two monkeys of equal size, one 'immune' ('I') and the other normal ('N') were anaesthetized with ether, their femoral vessels exposed, clamped and cannulated aseptically and sufficient heparin injected in both to render their blood non-coagulable. The artery of 'I' was then connected with the vein of 'N' and vice versa with short lengths of rubber tubing. The cannulae and rubber tubing having been filled with sterile physiological saline, the clamps on all the vessels were released simultaneously. A cross circulation between the two monkeys was thus established and maintained for more than 40 minutes. During this period 'I' bled into 'N' and 'N' into 'I', and a complete mixture of the bloods of the two animals was brought about. When the cross circulation was discontinued after 40 minutes, 'I' had 50 per cent of its own blood and 50 per cent of 'N's' blood and vice versa. The vessels were ligated at the end of the experiment and the wound sutured aseptically. The animals quickly recovered from the operation and as collateral circulation was rapidly established the animals were not inconvenienced as a result of the operation in any way.

When the cross circulation experiment was repeated with monkey 'I', using another normal monkey 'N<sup>1</sup>', 'I' had only 25 per cent of its own blood and 75 per cent of normal blood obtained from 'N' and 'N<sup>1</sup>'. This percentage of replacement could be improved by using monkeys of different sizes, but such experiments require careful calculation and experimental adjustments, for a bigger monkey would pump larger quantities of blood into the smaller monkey than the latter could tolerate. The percentage of replacement could also be increased by performing cross circulation more than twice.

#### CITRATE METHOD.

This method consisted in bleeding a monkey from an artery and transfusing citrated blood through its vein. By repeating this process several times, a desired replacement of blood can be effected. Thus, when two monkeys of approximately 4 kg. weight were bled separately into citrated saline, 70 c.c. being taken out of each and the blood of one transfused into the other, a complete mixture of the bloods of the two monkeys was brought about by repeating the process five times. The disadvantage of this method is that



with every transfusion a certain volume of citrated saline has to be injected, and the number of transfusions has, therefore, to be limited. However, a 50 per cent replacement can be achieved by this method also. The experiment can be repeated on the same animal next day and a further replacement brought about. The advantage of this method is that the amount of blood withdrawn and transfused can be accurately measured, and the degree of replacement of blood correctly ascertained.

### RESULTS.

Preliminary experiments were first made to see whether the induction and maintenance of ether anaesthesia and the operative procedures involved in both these methods affected the vitality of the parasites or the immunity of monkeys in any way. It was found that these were not influenced to any appreciable extent. The parasites maintained their vitality to the fullest extent and the degree of immunity was not affected in any way.

Tables I, II and III summarize the results obtained in three sets of experiments. In most of the experiments, the presence of immunity was tested by injecting a specified number of parasitized R. B. C's. from a freshly infected monkey. In the remaining, no further infection was found to be necessary, because the few parasites present in the 'immune' blood were sufficient to produce a severe infection. The number of parasites injected and the route of injections varied and are given in the tables.

TABLE I.

*Immunity in 'immune' monkeys whose blood was replaced by normal (non-immune) blood.*

Experiment number.	Percentage of blood replaced.	Number of parasites used for re-infection.	Route of re-infection	Result.	Immunity.
1	75	$1 \times 10^{10}$	I V	* Parasites disappeared within 48 hours	+
2	70	$1 \times 10^9$	"	"	+
3	70	$2 \times 10^9$	"	"	+
4	75	$1 \times 10^9$	"	"	+
5	75	$1 \times 10^9$	"	"	+
6	80	$2 \times 10^9$	"	"	+

Notes.—I.V. = Intravenous.

\* In this and subsequent tables, 'disappeared' means came to the level of chronic infection, i.e., one parasite in several fields.

In the experiments incorporated in Table I, the animals had their own, i.e., 'immune', cells and a varying amount of foreign 'non-immune' blood. It will be seen that even when nearly 80 per cent of the 'immune' blood of the animal was removed and replaced with normal blood (Experiment 6), the animal



could get rid of the freshly injected parasites as readily and effectively as any ordinary immune monkey having its own 'immune cells' and 'immune blood'. The parasite count immediately after the injection of fresh parasites in this experiment (Experiment 6) was 10 per 10,000 R. B. Cs., and it fell to the level of chronic infection (one parasite in several fields) within 48 hours and remained so for some months afterwards.

TABLE II.

*Immunity in normal monkeys whose blood was replaced by 'immune' blood.*

Experi- ment number.	Percentage of blood replaced.	Parasites in donor blood.	Number of para- sites used for re- infection	Route of re-infection	Result.	Immu- nity.
1	25	+	..	..	Died of infection due to parasites present in 'immune blood'.	-
2	25	+	..	..	Heavy infection; treated	-
3	25	-	$6 \times 10^5$	I.P. (after 2 weeks)	Died of infection	-
4	25	-	$2 \times 10^5$	"	"	-
5	33	+	..		Died of infection due to parasites in 'immune blood'.	-
6	50	+	..	.	"	-
7	50	+	..	..	"	-
8	50	-	$3 \times 10^5$	I.V. (after 2 weeks).	Parasites appeared increased and then disappeared.	+
9	50	-	$2 \times 10^5$	I.P. (after 2 weeks).	"	+
10	50	-	$2 \times 10^5$		Died of infection.	-
11	50	-	$2 \times 10^5$	"	"	-
12	50	+	..	..	Died of infection due to parasites in 'immune blood'.	-
13	75	+	$8 \times 10^5$	I.P. (same day).	Parasites appeared increased and disappeared.	+
14	85	+	..	..	"	+

Notes.—I.V. = Intravenous.  
I.P. = Intra-peritoneal.

+ = Parasites present.  
- = " absent.



It will be seen from the results given in Table II, that when the percentage of replacement is 75 or more, the monkeys acquire immunity, and that when it is only 33 per cent or less they fail to do so. If, however, only 50 per cent of the blood is replaced, the animals may or may not acquire immunity. Experiments 1 to 5 show that if 'immune blood' is present in a monkey to the extent of about one-fourth to one-third its total blood volume, this is not enough to prevent the progress of infection, the small number of parasites present in the 'immune blood' being sufficient to carry the infection to a fatal termination. If however, half the blood is 'immune blood' (Experiments 6 to 12) the animal may or may not acquire immunity. In all animals with 50 per cent replacement with 'immune blood' containing parasites, the small number of parasites present were sufficient to carry the infection to a fatal termination. In animals which had 50 per cent of their blood replaced by 'immune blood' free from parasites, some showed immunity towards superinfection and some did not. In those cases, however, where the replacement of normal blood with 'immune blood' was 75 per cent or more, evidence of immunity was obtained even though the 'immune blood' was not free from parasites. It should, however, be noted that in both the monkeys (Experiments 13 and 14), which had such high percentage of replacement, the parasites gradually increased in number for some days and then gradually decreased to the level of chronic infection. Thus, in Experiment 13 (Table II) where, in addition to the parasites originally present in the 'immune blood', an extra infection with  $8 \times 10^6$  parasites was given, the parasites were present in the peripheral blood on the third day of cross circulation; these gradually increased and on the sixth day they numbered 10, on the seventh day 30 and on the eighth day 80 per 10,000 R. B. Cs. From the ninth day onward, their number began to decline and later fell to the level of chronic infection. Similarly in Experiment 14 (Table II), with about 85 per cent replacement of normal blood with 'immune blood', the parasites were seen in the peripheral blood on the sixth day and their number gradually increased till on the tenth, eleventh and twelfth day they numbered 10, 80 and 100 respectively per 10,000 R. B. Cs. From the thirteenth day onward, the number began to decrease and gradually fell to the chronic infection level. These experiments, therefore, make it doubtful whether the 'immune blood' alone is sufficient to protect the animal from infection. The gradual increase and subsequent decrease of the number of parasites suggest that the 'immune blood' was acting indirectly, and that the cells had to play some part in this immunity and, further, that it required at least nine days contact with 'immune blood' to give the cells the necessary power to overcome the infection. This hypothesis is supported by Experiments 8 and 9 (Table II) where parasite-free 'immune blood', when in contact with normal cells for two weeks, was able to render the monkeys immune to fresh infection.

It was, therefore, decided to study the action of 'immune blood' in freshly infected monkeys having a moderately severe infection (about 1,000 parasites per 10,000 R. B. Cs.). This degree of infection was chosen because in the technique of cross circulation a large proportion of infected blood is removed and replaced with 'immune blood', so that the degree of infection is lessened, theoretically by 50 per cent, if 50 per cent blood is replaced, the number of parasites in 'immune blood' being considered as negligible. Practically, however, the reduction in the degree of infection is much greater in such experiments, possibly because the infected blood has to circulate through the 'immune cells' for approximately an hour and a large number of parasites are



destroyed by the 'immune cells' during this short period of one hour. Table III shows the results obtained in cross circulation experiments with 'infected' and 'immune' animals.

TABLE III.  
*Effect of replacing 'infected blood' with 'immune blood'.*

Experiment number.	Degree of infection before cross circulation (parasites per 10,000 R. B. Cs.).	Percentage of blood replaced.	Result.	Immunity.
1	1,700	50	Died of infection.	—
2	930	50	"	—
3	1,000	60	Chronic infection.	+
4	250	60	"	+
5	1,060	70	"	+

In these experiments, the number of parasites decreased within the first few days after cross circulation; thereafter, their number gradually increased and this was followed by a decrease in those animals which passed into the chronic stage. Experiment 5, where a replacement of 70 per cent of blood was effected, may be described to illustrate the results. On the eighth day after intra-peritoneal infection, the animal had a parasite count of 1,060 in the peripheral blood. On this day, 70 per cent of its blood was replaced with 'immune blood', and it had only 15 parasites per 10,000 R. B. Cs. after this replacement. The number of parasites was very small during the first two days after cross circulation, slightly increased on the third day, and from the fourth to the tenth day (after cross circulation) the parasite count was 50, 100, 120, 300, 80, 20 and 5 respectively per 10,000 R. B. Cs. From the eleventh day onward the animal passed into the stage of chronic infection.

It was not possible to ascertain to what the initial disappearance of the parasites was due. Control experiments showed that neither the anaesthesia nor the mechanical interference with blood which was involved in the cross circulation technique was responsible for this. The initial disappearance of the parasites would, therefore, indicate that the 'immune blood' had a direct action on the parasites. The possibility, however, exists that the initial infection had already set the cellular mechanism in action, and the presence of an adequate quantity of 'immune blood' sufficed to stimulate the cells to play their part. This, however, would not explain why the parasites increased from the fourth to the seventh day after cross circulation (twelfth to fifteenth day after infection).

#### DISCUSSION.

The experimental details presented in Tables I, II and III do not permit of straightforward conclusions being drawn. The results incorporated in Table I



were very uniform, and in none of the experiments was there any indication to show that immunity in these six monkeys had changed to any appreciable extent, because their 'immune blood' was removed and replaced with normal 'non-immune blood'. It has not been possible to achieve a cent per cent replacement of the blood of the immune animal with normal blood, and, therefore, the conditions of Mulligan and his co-workers (*loc. cit.*) that 'a full measure of one factor with an adequate measure of the other' were still operating. The actual response of the animals to fairly heavy fresh infection, however, was so quick and complete that one is led to believe that even if a cent per cent replacement of blood had been possible, the animals would retain their immunity to the full extent. Whether this degree of immunity would persist after a lapse of some weeks after 'immune blood' is replaced with normal blood is a point still under investigation. Table I, however, shows the important part played by the cells in giving immunity to the animals.

The fact that antibody concentration in serum varies in Rhesus monkeys with *P. knowlesi* infection is now recognized by several workers. This may, therefore, explain the varying results of the experiments summarized in Table II. The fact that parasite-free immune blood could confer immunity to normal animals shows that it is not the parasites, but the antibodies present in the blood, that are responsible for producing immunity. A 50 per cent replacement with 'immune blood' may or may not give immunity to normal monkeys, the action being possibly determined by the concentration of humoral antibodies in the replacing 'immune blood'. A higher replacement gave immunity, but perhaps there was a time factor operating, which suggests that the part played by humoral antibodies was indirect, i.e., through stimulation of the cells. The direct effect of these antibodies is suggested by experiments detailed in Table III. As pointed out above, however, the rôle played by cells in such experiments is still obscure. In general, therefore, one can conclude that in malarial immunity in the Rhesus monkey against *P. knowlesi* infection, both the 'cellular' and 'humoral' factors are working together in close co-operation. The part played by the cellular agencies is important and direct. The humoral agencies probably act indirectly by stimulating the cells and not directly on the malarial parasites.

### SUMMARY AND CONCLUSIONS.

(1) Methods of replacing the blood of a monkey with that of other monkeys are described and were used to study the rôle played by 'cellular' and 'humoral' agencies in immunity in monkey malaria.

(2) When about 80 per cent of the blood of an immune monkey was withdrawn and replaced with normal non-immune blood, the monkey retained its immunity against heavy superinfection.

(3) When the blood of a normal monkey was withdrawn and replaced with 'immune blood', the normal monkey acquired immunity, if a sufficiently high (more than 70 per cent) replacement was effected.

(4) When the blood of an infected monkey was replaced with 'immune blood', the infected monkey got rid of the infection and passed into a stage of chronic infection, if a sufficient amount of blood had been replaced.

(5) The findings mentioned above are discussed, and it has been suggested that cellular agencies play an important and direct part in malarial



immunity, whilst the humoral agencies probably play an indirect rôle by stimulating the cellular mechanism.

#### ACKNOWLEDGMENT.

The author wishes to thank Lieut.-Colonel S. S. Sokhey, I.M.S., Director, Haffkine Institute, for his interest in this work.

#### REFERENCES.

- COGGESHALL, L. T., and KUMM, H. W. *J. Exp. Med.*, **66**, p. 177.  
(1937).  
DIKSHIT, B. B., and GANAPATHI, K. *J. Mal. Inst. Ind.*, **3**, p. 525.  
(1940).  
MULLIGAN, H. W., SOMMERVILLE, T., *Ibid.*, **3**, p. 563.  
and SWAMINATH, C. S. (1940).  
TALIAFERRO, W. H., and MULLIGAN, *Ind. Med. Res. Mem.*, No. **29**.  
H. W. (1937).



## ON THE ABDOMINAL TERGITES OF ADULT MANSONIOIDES THEOBALD, 1907.

(DIPTERA, CULICIDÆ)

BY

M. A. U. MENON

(Entomological Assistant, Public Health Laboratory, Central Research Institute,  
Travancore, Truvandrum).

[August 12, 1941]

THE abdominal segments in the females of the subgenus *Mansonioides* Theobald, 1907, are in many ways peculiar as they have certain characteristic features not so far found in other species of mosquitoes. On the tergite of the eighth abdominal segment, there occurs a comb of stout, thick and heavily chitinized teeth, to which attention was first drawn by Bonne-Wepster (1930). Edwards (1930) showed that the apex of the eighth sternite is produced into two lateral lobes which are feebly chitinized. The writer describes in this paper certain other features which he has observed in the abdominal segments of *Mansonioides*, besides the two characters mentioned above. Four species of *Mansonioides* were studied in this connection, namely, *M. annulifera* (Theobald), *M. uniformis* (Theobald), *M. indiana* Edwards and *M. longipalpis* (van der Wulp). The first three species were studied from material collected in India and the last, from that collected in Java.

The following are the two additional characters noted in regard to the species studied:—

(i) The abdominal tergites 4 to 7 in the female show heavy chitinization along their anterior border\*.

(ii) The anterior and posterior borders of tergites 6 and 7 are concave, so that the tergites are narrower about the middle than near the sides.

### CHITINOUS THICKENINGS ON THE TERGITES.

The thickenings on the anterior part of the tergites are very clearly seen in preparations cleared with caustic potash. But even live, engorged females, when

---

\* In *Mansonioides annulifera*, the chitinization occurs only on tergites 6 and 7 (*vide infra*).



viewed under low magnification, show these thickenings fairly well. In unfed mosquitoes, as the abdominal segments are telescoped, it is difficult to see the anterior border of the tergites distinctly. The males do not possess these thickenings.

In *M. indiana*, *M. uniformis* and *M. longipalpis*, the chitinous thickenings occur on tergites 4 to 7 (Plate XXIV, figs. 2, 3 and 4), whereas in *M. annulifera*, they are present only on tergites 6 and 7 (Plate XXIV, fig. 1). Normally the thickenings on tergites 4 and 5 are broader and more pronounced than those on tergites 6 and 7, and often have a median extension posteriorly. The chitination on tergite 7 is usually more conspicuous than that on tergite 6.

The majority of wild females examined show the thickenings, but they are absent in young and freshly bred out specimens. The photomicrographs in Plate XXV illustrate this difference as seen in young and old mosquitoes of two species of *Mansonioides*, namely, *uniformis* (figs. 5 and 6) and *annulifera* (figs. 7 and 8).

Some observations on the development of these thickenings were carried out with bred out adults of *M. uniformis*, of different ages varying from 1 to 19 days\*. The results of these observations are summarized in the table.

It can be seen from the table that the development of the thickenings is more or less gradual, and progressive with age. They are first faintly demarcated as light, yellowish-brown ridges near the anterior border of the tergites. These ridges become thicker and darker as the mosquito grows, till they become dark-brown in colour and quite distinct. It is usually the thickening on the seventh tergite that appears first, followed by those on the fourth and fifth tergites. The thickening on the sixth tergal plate appears last. In the series studied, a minimum of 6 days was found to be necessary for the thickening on the seventh tergite to appear, and a minimum of 8 days for the thickenings on all the tergites 4 to 7 to develop. But the minimum period required for all the thickenings to acquire their characteristic shape and dark-brown colour was 13 days. In some specimens, however, the development of the thickenings was observed to be very much delayed. For instance, as the table shows, one specimen had developed only the thickening of the seventh tergite on the 11th day, and another on the 15th day after hatching. But in the majority of cases the development of the thickenings started before the mosquitoes were a week old, and was complete before they were 3 weeks old.

#### SHAPE OF TERGITES 6 AND 7.

In all the four species of *Mansonioides* studied, the anterior and posterior borders of tergites 6 and 7 are concave, so that a median constriction occurs on these plates (Plate XXIV, figs. 1 to 4). Generally, this feature is more conspicuous in the seventh tergal plate than in the sixth.

These two features, namely, the chitinous thickenings along the anterior border of the posterior abdominal tergites and the peculiar shape of the sixth and seventh tergites, have not been observed in any of the other genera of mosquitoes studied, e.g., *Culex*, *Aedes*, *Ficalbia* and *Anopheles*. It would appear, therefore, that they are characteristic of *Mansonioides*.

---

\* The mosquitoes were kept alive on raisins and water. They were not fed on blood.







EXPLANATION OF PLATE XXIV.

Fig. 1. Abdomen of a female *Mansonioides annulifera* showing chitinous thickenings at the bases of tergites 6 and 7.

Figs. 2, 3 and 4. Abdomen of female *M. longipalpis*, *M. indiana* and *M. uniformis* respectively, showing thickenings at the bases of tergites 4, 5, 6 and 7.

*N.B.*—The drawings are of preparations cleared with caustic potash solution.



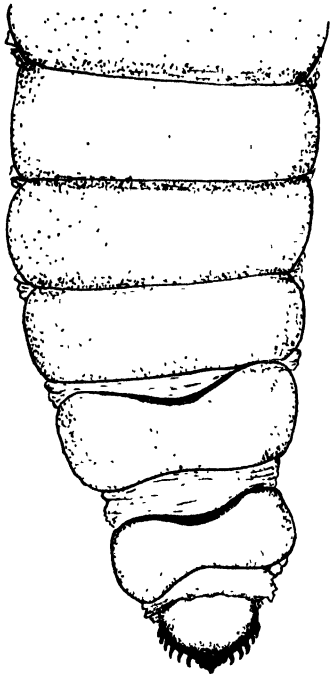


Fig. 1.

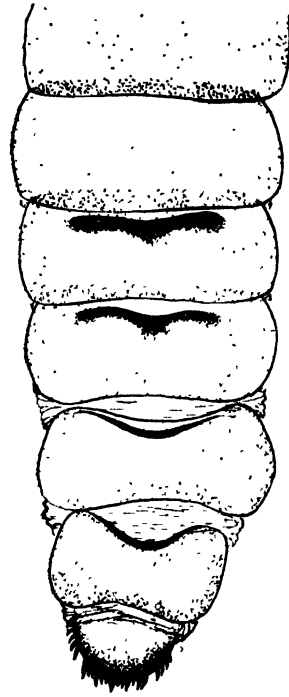


Fig. 2.

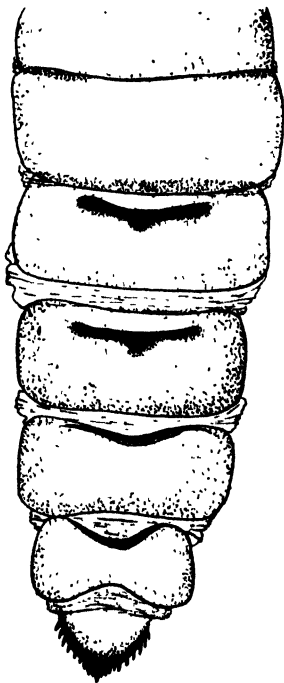


Fig. 3.

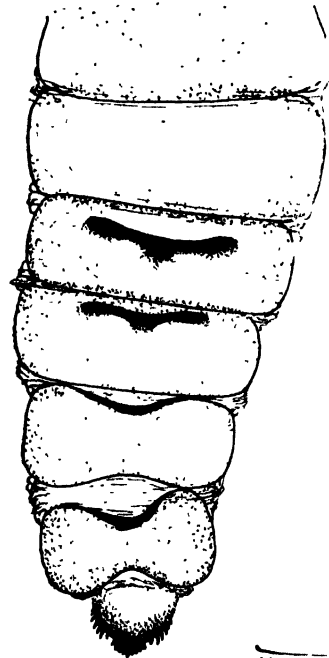


Fig. 4.

*MENON*



PLATE XXV.



Fig. 5.  
(50 X)



Fig. 6.  
(50 X)



Fig. 7.  
(50 X)



Fig. 8.  
(50 X)



EXPLANATION OF PLATE XXV.

- Fig. 5. Abdomen of a female *M. uniformis*, aged 14 days, showing well-developed chitinous thickenings on tergites 4 to 7.
- „ 6. Abdomen of a female *M. uniformis*, aged 3 days, in which the thickenings have not developed.
- „ 7. Abdomen of a female *M. annulifera*, aged 20 days, in which the thickenings have developed on tergites 6 and 7.
- „ 8. Abdomen of a female *M. annulifera*, aged 3 days, in which the thickenings have not developed.

*N.B.*—The photomicrographs are of preparations cleared with caustic potash solution. (Magnification 50 ×).







TABLE.

*Relation between age and development of chitinous thickenings on the abdominal tergites of Mansonoides uniformis.*

Age in days.	Number of specimens examined.	Number of specimens with no thickenings.	Number of specimens with thickening on tergite 7 only.	Number of specimens with thickenings on tergites 4 and 7 only.	Number of specimens with thickenings on tergites 4, 5 and 7 only.	NUMBER OF SPECIMENS WITH THICKENINGS DEVELOPED ON TERGITES 4, 5, 6 AND 7.	
						With heavy thickening on tergite 7 only. Others light*.	With heavy thickenings† on all four tergites.
1	5	5	..	..	..	..	..
2	6	6	..	..	..	..	..
3	6	6	..	..	..	..	..
4	5	5	..	..	..	..	..
5	4	4	..	..	..	..	..
6	4	1	3	..	..	..	..
7	5	..	5	..	..	..	..
8	5	..	2	1	1	1	..
9	11	..	.	..	6	5	..
10	7	..	..	2	5	..	..
11	9	..	1	2	4	2	..
12	5	..	..	1	2	2	..
13	7	..	..	..	1	4	2
14	19	.	..	2	8	6	3
15	7	..	1	..	2	1	3
16	13	..	..	2	..	6	5
17	16	..	..	..	4	3	9
18	3	..	..	..	..	..	3
19	5	..	..	..	..	..	5

Notes.—\* Light thickenings are yellowish brown.

† Heavy thickenings are of a dark-brown colour.



## SUMMARY.

In four species of *Mansonioides* studied, in addition to the characters described by Bonne-Wepster (*loc. cit.*) and Edwards (*loc. cit.*), namely, the occurrence of a comb of teeth on the eighth tergite and two lateral lobes on the eighth sternite of the females, it is observed that a chitinous thickening occurs on tergites 4 to 7 in *M. indiana*, *M. uniformis* and *M. longipalpis*, but only on tergites 6 and 7 in *M. annulifera*. In all these four species, the shape of the tergal plates 6 and 7 is characteristic in that the anterior and posterior borders of these plates are concave. These features have not been observed in any of the other genera of mosquitoes studied.

## ACKNOWLEDGMENTS.

The author thanks the authorities of the Central Research Institute, University of Travancore, for their permission to publish this paper. He is also thankful to Rajyasevanirata Dr. C. O. Karunakaran, Superintendent, Public Health Laboratory, Trivandrum, for the interest he has shown in this work, and to Dr. M. O. T. Iyengar for helpful criticism and for kindly supplying the author with specimens of *M. annulifera* and *M. longipalpis*.

## REFERENCES.

- |                          |  |
|--------------------------|--|
| BONNE-WEPPER, J. (1930)  | .. <i>Meded. Volks. Ned. Ind.</i> , <b>19</b> , Part I, pp. 196-212. |
| EDWARDS, F. W. (1930) .. | .. <i>Bull. Ent. Res.</i> , <b>21</b> , pp. 541-545.                 |



ON THE COMPARATIVE EFFICACY AND RELATIVE COSTS  
OF BIOLOGICAL AND CHEMICAL METHODS OF  
MOSQUITO CONTROL IN CLEAN-WEEDED  
RAILWAY BORROWPITS AT  
FULESHWAR, BENGAL\*.

BY

T. J. JOB, D.Sc.

*Lady Tata Memorial Research Scholar.*

*(From the Laboratories of the Zoological Survey of India, Calcutta.)*

[August 27, 1941.]

INTRODUCTION.

THE present paper is a continuation of work already reported on the mosquitocidal values of indigenous larvivorous fishes (Job, 1940*a*; 1940*b*; 1941*a*; 1941*b*), and deals with experiments conducted in railway borrowpit sections at Fuleshwar in the Hooghly Delta Section of the Bengal-Nagpur Railway to determine the comparative efficacy and relative costs of mosquito control with *Aplocheilus panchax* (Hamilton) and paris green.

EXPERIMENTS.

Bunds were erected in a borrowpit north of the railway bridge between Fuleshwar and Ulubaria dividing it into three equal sections *a*, *b* and *c*, each 20 feet by 12 feet. The ecological factors were more or less similar in the three sections. The water was slightly turbid and of an average depth of 2 feet. The margin was clean and the bottom muddy. Vegetation clearing being part of the routine followed in the Bengal-Nagpur Railway borrowpits where paris green is used for larval control, the flora was poor, and consisted mainly of diatoms and patches of algæ. Young frogs, snails, larvæ of mayflies, dragonflies, chironomids and other insects appeared in the water. The experiments proper were started on September 27, 1940, when the larval densities in the sections were fairly high (for technique of determining larval density see Job, 1941*a*). Section *a* was treated with paris green, fish were introduced into section *b*, and section *c* was left untreated to serve as control.

Section *a* was treated with paris green on every fifth day, according to the routine followed by the antimalaria staff in the neighbouring control areas.

---

\*Part of the thesis approved for the degree of Doctor of Science by the University of Madras.



## 212 *Efficacy and Relative Costs of Methods of Mosquito Control.*

A rotary blower was used, and 4 ounces of paris green mixture of 2 per cent strength was used on each occasion. Densities of the different aquatic stages of anophelines and culicines were recorded before applying paris green, and on alternate days thereafter. It was found that all late instar larvæ of anophelines disappeared after the application of paris green, but the earlier instars were not affected. The pupæ, of course, were unaffected. In the case of culicines, all stages were unaffected by paris green. These results confirm the observations of other workers (Covell, 1935, p. 35). As a result of discontinuing the application of paris green on a certain date (*vide* Table, 5.x.40), even the anophelines began to thrive, and on 9.x.40 section *a* was found to harbour all stages of anophelines as well as culicines.

Into section *b*, 96 adult and 48 young specimens of *Aplocheilus panchax* were introduced from a nearby hatchery in accordance with the ratio between the actual area to be controlled and the number of fish required (Job, 1941a). It may, however, be noted that the breeding of mosquitoes was practically confined to a strip of water about 9 inches wide along the margins. Larval densities were recorded before the introduction of fish and on alternate days thereafter. The late instar larvæ and pupæ were the first to disappear, followed by the early instars. Both anophelines and culicines were preyed upon, and the section was entirely free of mosquito larvæ by 1.x.40, and continued to remain so thereafter.

In the comparison section *c*, all stages of anophelines and culicines continued to thrive, exhibiting natural fluctuations in their densities.

The details of observations are recorded in the table. From the above field experiments it can be inferred that:—

- (i) Paris green is destructive to anopheline larvæ of the late instars only, other stages of anophelines and all stages of culicines being unaffected.
- (ii) *Aplocheilus panchax* destroys all aquatic stages of both anophelines and culicines.

### RELATIVE COSTS.

The following statements calculated for one year give an approximate idea of the relative costs of the measures under the conditions prevailing in the Hooghly Delta Section of the Bengal-Nagpur Railway:—

#### I. *Cost of treatment with paris green of borrowpit section 'a' excluding the cost of the supervising staff.*

	Rs.	As.	P.
(i) Price of 12½ lb. of paris green mixture (2 per cent, volumetric) for 50 applications during 8 months of the year, six times a month .. .. .	1	1	0
(Owing to the war the price of paris green has greatly increased.)			
(ii) Cost of trained labour for 4½ working days at 10 annas a working day (taking 30 minutes for each application, and 6 hours of intensive work being reckoned for a normal working day) ..	2	9	8
(iii) Proportionate depreciation in the cost of the machinery (rotary blower, mixer, etc.) ..	0	2	4
<b>TOTAL</b> ..	<b>3</b>	<b>13</b>	<b>0</b>



TABLE.

*Details of densities of aquatic stages of anophelines and culicines and operations in the railway borrowpit sections 'a', 'b' and 'c' on various dates.*

Date of observation.	Section.	ANOPHELINEs.						CULICINEs.						Total mosquito stages.	Operations.
		Larval instars.				Pupæ.	Total.	Larval instars.				Pupæ.	Total.		
		1st	2nd	3rd	4th			1st	2nd	3rd	4th				
27.ix.40	a	1.3	1.6	2.0	1.2	0.9	7.0	0.5	1.2	0.8	0.6	0.5	3.6	10.6	Paris green applied after noting the densities.
	b	1.0	1.7	2.3	1.6	1.0	7.6	0.7	0.8	1.2	0.9	0.5	4.1	11.7	Ninety-six adult and 48 young specimens of <i>A. panchar</i> introduced after noting the densities.
	c	0.8	0.5	1.2	1.0	0.5	4.0	1.2	0.0	0.8	1.6	0.9	4.5	8.5	Left as comparison.
29.ix.40	a	1.9	0.7	0.0	0.0	0.0	2.6	1.1	1.4	1.6	0.9	0.7	5.7	8.3	..
	b	0.7	0.4	0.2	0.0	0.0	1.3	0.6	0.4	0.0	0.0	0.0	1.0	2.3	..
	c	0.9	1.1	1.2	1.5	0.7	5.4	1.1	0.7	0.4	1.5	0.7	4.4	9.8	..
1.x.40	a	1.4	1.5	0.9	0.0	0.0	3.8	0.9	1.2	1.3	1.6	0.3	5.3	9.1	Paris green applied after noting the densities.
	b	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	..
	c	0.2	1.2	0.9	1.5	1.0	4.8	0.8	1.6	0.7	1.1	1.0	5.2	10.0	..
3.x.40	a	0.7	1.2	0.0	0.0	0.0	1.9	1.1	1.1	1.5	0.4	0.2	4.3	6.2	..
	b	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	..
	c	0.8	1.0	0.8	1.3	0.4	4.3	0.7	1.3	1.0	1.7	0.3	5.0	9.3	..
5.x.40	a	0.8	1.6	1.0	0.0	0.0	3.4	0.9	1.3	1.2	0.6	0.1	4.1	7.5	..
	b	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	..
	c	0.6	1.4	1.3	0.9	0.6	4.8	0.7	1.6	1.8	1.5	0.8	6.4	11.2	..
9.x.40	a	0.5	1.5	1.8	0.9	0.2	4.9	0.6	0.9	1.4	0.8	0.2	3.9	8.8	..
	b	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	..
	c	0.3	1.5	1.0	1.2	0.3	4.3	0.9	1.3	2.0	1.1	0.5	5.8	10.1	..



## 214 *Efficacy and Relative Costs of Methods of Mosquito Control.*

### II. *Cost of control by fish of borrowpit section 'b' of similar area and ecology as section 'a'.*

		Rs.	As.	P.
(i)	Cost of gathering 144 specimens of <i>Aplocheilus</i> <i>panchax</i> and introducing them into the section ..	0	3	0
(ii)	Cost of replenishing, if needed ..	0	3	0
	TOTAL ..	0	6	0

It is evident that even excluding the expenditure on the supervising staff so essential in chemical control, the cost of introducing and maintaining fish for larval control means a saving of over 90 per cent as compared to the cost of treatment with paris green. (For discussions on the limitations of applying paris green, see Covell, *loc. cit.*, pp. 34, 35; Missiroli, 1927; Chatterji, 1934, p. 23; and Sen, 1939, pp. 9-12.)

### SUMMARY.

Field experiments conducted in railway borrowpit sections in Howrah District, Bengal, with *Aplocheilus panchax* (Hamilton) and paris green, have shown that the fish destroys all aquatic stages of anopheline as well as culicine mosquitoes, while paris green is destructive to the late instar larvæ of anophelines only. The relative costs of applying paris green and of introducing and replenishing supplies of fish in experimental borrowpit sections have been estimated, and it is found that the latter is over 90 per cent cheaper than the former.

### ACKNOWLEDGMENTS.

The author wishes to thank Dr. Bains Prashad, Director, Zoological Survey of India, for facilities to work in the laboratories of the Zoological Survey of India and for helpful criticism; Rai Bahadur Dr. S. L. Hora for guidance and encouragement; Mr. A. Duncan, C.I.E., Agent and General Manager, Bengal-Nagpur Railway, for travelling facilities; and Mr. R. Senior White, Malariologist, Bengal-Nagpur Railway, for field facilities, and useful suggestions.

### REFERENCES.

- CHATTERJI, G. C. (1934) .. *Biological control of malaria in rural areas of Lower Bengal*, pp. 1-28.
- COVELL, G. (1935) .. *Anti-mosquito measures with special reference to India*. Malaria Bureau, No. 3, *Health Bulletin*, No. 11, pp. 1-60. (Fourth edition.)
- JOB, T. J. (1940a) .. On the breeding and development of Indian 'Mosquito-fish' of the genera *Aplocheilus* McClelland and *Oryzias* Jordan and Snyder. *Rec. Ind. Mus.*, **42**, pp. 51-79.
- Idem* (1940b) .. Notes on the geographical distribution and larvicidal propensities of *Horaichthys setnai* Kulkarni. *Jour. Bomb. Nat. Hist. Soc.*, **42**, pp. 201-202.
- Idem* (1941a) .. Efficiency of the Killifish, *Aplocheilus panchax* (Hamilton) in the control of mosquitoes. *Proc. Nat. Inst. Sci. Ind.*, **7**, pp. 317-350.



- JOB, T. J. (1941b) .. .. Food and feeding habits of the Glassfishes (*Ambassis Cuv and Val*) and their bearing on the biological control of guinea-worm and malaria. *Ind. J. Med. Res.*, **29**, pp. 851-862.
- MISSIROLI, A. (1927) .. .. Practical malaria prevention. *Riv. Malariol.*, **6**, 3, pp. 501-572.
- SEN, P. (1939) .. .. Can paris green kill fish? *Bengal Pub. Hlth. Jour.*, **1**, pp. 9-12.







# STUDIES ON THE BEHAVIOUR OF *ANOPHELES MINIMUS*.

## Part V.

### THE BEHAVIOUR OF ADULTS IN RELATION TO FEEDING AND RESTING IN HOUSES.

BY

R. C. MUIRHEAD THOMSON.

(*Research Worker supported by the Royal Society and the London School of Hygiene and Tropical Medicine.*)

[September 10, 1941.]

## CONTENTS.

	PAGE.
INTRODUCTION .. .. .	218
METEOROLOGICAL CONDITIONS .. .	218
DAYTIME RESTING PLACES .. .. .	219
CLIMATIC CONDITIONS INSIDE COOLIE HOUSES . . .	223
TEMPERATURE CONDITIONS IN COWSHEDS .. .	225
LIGHT INTENSITY IN RESTING PLACES .. .. .	225
DIGESTION OF BLOOD AND MATURATION OF OVARIES .. .	226
COMPOSITION AND SIZE OF POPULATION IN HOUSES .. .	227
REACTIONS OF <i>A. minimus</i> TO LIGHT AT SUNRISE AND SUNSET .. .	229
MARKING EXPERIMENTS .. .. .	231
NOCTURNAL ACTIVITY AND TIME OF FEEDING .. .	233
NUMBER OF EGGS LAID AT DIFFERENT SEASONS .. .	235
MATING AND FERTILIZATION .. .. .	235
FEEDING OF ANIMALS .. .. .	236
FEEDING ACTIVITIES IN THE COLD WEATHER .. .	238
REACTIONS OF <i>A. minimus</i> TO HUMIDITY .. .	239
REACTIONS OF <i>A. minimus</i> TO TEMPERATURE .. .	239
THERMAL DEATH POINT OF ADULTS .. .. .	241
LABORATORY EXPERIMENTS ON LONGEVITY OF <i>A. minimus</i> .. .	241
SUMMARY .. .. .	244
REFERENCES .. .. .	244



## INTRODUCTION.

IN the previous papers of this series (Thomson, 1940), the author has been principally concerned with the behaviour of the mosquito in relation to the ecology of the breeding place. In the present publication, some other aspects of behaviour are dealt with, namely the activity of *A. minimus* in houses in relation to feeding and resting. This subject does not lend itself so well to systematic investigation by experimental methods, and most of the material in this paper consists of straightforward and often disconnected observations which have accumulated during the last three years.

Many activities of *A. minimus* have been briefly investigated and described, and no attempt has been made to follow up any of these aspects of behaviour in great detail. As a result, a number of the observations are necessarily incomplete, but it is hoped that the sum total of the results will help to give a clearer idea of feeding and resting activities, and to advance our knowledge of *A. minimus* towards the standard of what should be known about such an important vector of malaria.

Most of the work was carried out in the Assam Valley, but some attention was also paid to conditions in the Dooars of North Bengal.

## METEOROLOGICAL CONDITIONS.

The meteorological conditions at Tocklai Experimental Station in the Sibsagar District of Upper Assam are shown in Tables I and II, in which the temperature and humidity conditions for each month from 1936 to 1940 are recorded, and the monthly rainfall from 1918 to 1941. The most outstanding features of the climate which are likely to affect mosquitoes are as follows:— There is a distinct cold weather with the mean minimum temperature below 60°F. (15.5°C.) for 4 months, and with the minimum temperature frequently falling below 45°F. (7.2°C.) for one or two weeks in the coldest time of the year. In the Assam Valley, there is no well-marked hot dry season in March and April, although this becomes more conspicuous in the Dooars and Darjeeling Terai of North Bengal. The temperature rises gradually till about June, and for the following three or four months there are fairly uniform conditions of high temperature and high humidity. While the absolute humidity during that period is constantly high—frequently exceeding 1 inch—the relative humidity shows a considerable range each day, and may vary from 100 per cent in the early morning to 60 per cent or lower in the afternoon of the same day.

Reference to the rainfall records shows considerable fluctuation in the amount and distribution even in the same locality. In each of the three years in which the present investigation was made, the annual rainfall was below the average for the previous 20 years, 1918 to 1937, by 3.38, 20.42 and 12.88 inches respectively. The year 1939 was the driest on record, and this was followed by another dry year with rainfall well below the average. The occurrence of two such dry years together had an unfavourable effect on the investigation, and by the latter part of 1940 larvæ of *A. minimus* were so scarce and scattered that no field experiments could be carried out. This erratic rainfall undoubtedly has a great influence on the population of *A. minimus*, but records would have



to be studied for many more years before one could work out the exact relation between the two.

### DAYTIME RESTING PLACES.

Although a very large number of catches and dissections of *A. minimus* have been recorded from Assam and North Bengal, the present author was surprised to find that the majority of these adult collections were made at night or in the very early morning, and that very little was known about the daytime resting places of this mosquito. In the Philippines, the daytime resting places of *A. minimus* var. *flavirostris* are mainly outside, in dark-shaded ravines and nullahs (Russell, 1931; 1932), while in Indo-China (Toumanoff, 1936) and Yunnan (Chang, 1940) *A. minimus* is evidently a domestic species resting in dark houses by day. In the tea garden districts of Assam, careful daytime searches soon showed that here also *A. minimus* is a domestic species, sheltering in dark houses and coolie huts by day. In many districts, particularly those in which effective control methods were in operation, it was extremely difficult to find adults of the species in the coolie lines by day, but the very large numbers recorded from other favourable districts, especially where control was imperfect or absent, left no doubt about the nature of the resting places.

The majority of the tea garden coolie houses investigated in Assam had bamboo and mud plaster walls with thatched roofs. Most houses were divided into two small rooms, in the darker of which most of the adult specimens of *A. minimus* were found. In all houses, the most favoured resting site was very similar, and an experienced collector knew at once the best places for catching. While many mosquitoes were found resting on the dark walls or on dark umbrellas and hanging clothes, by far the greater number were found hanging from the underside of bamboo beds, under piles of firewood supported on a bamboo framework, and similar dark horizontal surfaces. The ideal site was under one of the large bamboo *chang* beds, which may take up half of the dark room and usually remain in contact with two or three of the walls.

All the resting places were characterized by being easily accessible, and while mosquitoes were usually absent from the well-lit room, they were not particularly attracted to completely dark holes or corners in the dimly-lit room nor to the dark inside of pots or cooking vessels. They are frequently found settled on the dark earth floor under the *chang* bed, and in fact the great majority of mosquitoes are found in the lower half of the room. In the Dooars many of the huts have thatch walls, and in the dark corners of such houses specimens of *A. minimus* may be found resting on clean fresh thatch but seldom on old thatch covered with dusty cobwebs; they also show no tendency to burrow into thatch or hide in deep cracks or holes. Where mosquito-nets of inferior quality are used by the occupants of the house, specimens of *A. minimus* may often be trapped in these after feeding, and can be easily caught on the following days. In well-illuminated houses or rooms, this may be the only place in which this species is found.

The favourite resting place was much the same throughout the year, both in the cold weather and in the monsoon season. In many coolie lines, a striking feature was the patchy distribution of *A. minimus* in houses, some houses regularly yielding unusually large numbers of mosquitoes compared with others, probably due to their position or to the fact that they possessed specially suitable daytime resting places; and two or three such houses produced nearly



TABLE I.  
*Meteorological figures taken at Tocklai Experimental Station, Upper Assam.*  
*[Mean maximum and minimum temperatures, vapour tension, and relative humidity (8 a.m.)]*

		1936.	1937.	1938.	1939.	1940.
January	Max.	70.68°F. (21.5°C.)	70.87°F. (21.6°C.)	72.33°F. (22.4°C.)	73.99°F. (23.3°C.)	74.01°F. (23.3°C.)
	Min.	46.32°F. (7.9°C.)	45.11°F. (7.3°C.)	49.70°F. (9.8°C.)	49.09°F. (9.5°C.)	45.85°F. (7.5°C.)
	V. T.	0.388 inch	0.367 inch	0.423 inch	0.427 inch	0.360 inch
	R. H.	97.09 per cent	97.97 per cent	98.81 per cent	98.32 per cent	96.93 per cent
February	Max.	73.01°F. (22.7°C.)	74.23°F. (23.5°C.)	76.15°F. (24.5°C.)	74.96°F. (23.8°C.)	74.90°F. (23.8°C.)
	Min.	52.22°F. (11.2°C.)	53.91°F. (12.2°C.)	51.83°F. (11.0°C.)	54.52°F. (12.5°C.)	51.81°F. (11.0°C.)
	V. T.	0.449 inch	0.479 inch	0.459 inch	0.493 inch	0.458 inch
	R. H.	96.24 per cent	96.39 per cent	93.71 per cent	95.35 per cent	95.03 per cent
March	Max.	83.73°F. (28.7°C.)	82.25°F. (27.9°C.)	78.75°F. (25.9°C.)	83.81°F. (28.8°C.)	75.79°F. (24.3°C.)
	Min.	59.13°F. (15.0°C.)	57.75°F. (14.3°C.)	61.96°F. (16.6°C.)	58.80°F. (14.9°C.)	57.86°F. (14.3°C.)
	V. T.	0.552 inch	0.529 inch	0.610 inch	0.541 inch	0.519 inch
	R. H.	82.61 per cent	83.77 per cent	92.68 per cent	82.65 per cent	92.32 per cent
April	Max.	83.94°F. (28.8°C.)	87.29°F. (30.7°C.)	85.30°F. (29.6°C.)	82.46°F. (28.0°C.)	85.59°F. (29.7°C.)
	Min.	65.67°F. (18.7°C.)	65.63°F. (18.7°C.)	66.76°F. (19.3°C.)	65.15°F. (18.4°C.)	65.26°F. (18.5°C.)
	V. T.	0.683 inch	0.687 inch	0.715 inch	0.652 inch	0.660 inch
	R. H.	87.10 per cent	84.73 per cent	87.70 per cent	87.00 per cent	83.93 per cent
May	Max.	87.66°F. (30.9°C.)	88.00°F. (31.1°C.)	88.99°F. (31.6°C.)	87.16°F. (30.6°C.)	85.51°F. (29.7°C.)
	Min.	71.50°F. (21.9°C.)	70.04°F. (21.1°C.)	72.78°F. (22.6°C.)	71.22°F. (21.8°C.)	70.73°F. (21.5°C.)
	V. T.	0.814 inch	0.808 inch	0.852 inch	0.808 inch	0.817 inch
	R. H.	86.97 per cent	85.26 per cent	87.39 per cent	97.68 per cent	93.29 per cent
June	Max.	90.53°F. (32.5°C.)	91.40°F. (33.0°C.)	89.50°F. (31.9°C.)	88.23°F. (31.2°C.)	88.69°F. (31.5°C.)
	Min.	74.91°F. (23.8°C.)	74.98°F. (23.9°C.)	75.69°F. (24.2°C.)	75.04°F. (23.9°C.)	75.07°F. (23.9°C.)
	V. T.	0.890 inch	0.907 inch	0.945 inch	0.909 inch	0.929 inch
	R. H.	82.63 per cent	84.40 per cent	83.63 per cent	91.53 per cent	92.23 per cent
July	Max.	90.73°F. (32.6°C.)	91.48°F. (33.0°C.)	89.39°F. (31.8°C.)	90.11°F. (32.3°C.)	89.10°F. (31.7°C.)
	Min.	76.28°F. (24.6°C.)	76.17°F. (24.5°C.)	75.03°F. (23.9°C.)	76.02°F. (24.4°C.)	76.00°F. (24.4°C.)
	V. T.	0.938 inch	0.999 inch	0.930 inch	0.942 inch	0.935 inch
	R. H.	88.97 per cent	85.42 per cent	87.39 per cent	91.45 per cent	92.51 per cent



August	..	Max.	90-30°F. (32-4°C.)	89-84°F. (32-1°C.)	89-46°F. (31-9°C.)	90-67°F. (32-6°C.)	91-16°F. (32-8°C.)
	..	Min.	76-29°F. (24-6°C.)	75-90°F. (24-4°C.)	75-87°F. (24-3°C.)	76-03°F. (24-4°C.)	76-41°F. (24-6°C.)
	..	V. T.	0-948 inch	0-969 inch	0-949 inch	0-949 inch	0-963 inch
	..	R. H.	90-32 per cent	93-58 per cent	89-23 per cent	87-74 per cent	91-51 per cent
September	..	Max.	89-62°F. (32-0°C.)	88-98°F. (31-6°C.)	89-18°F. (31-7°C.)	88-43°F. (31-3°C.)	88-07°F. (31-1°C.)
	..	Min.	75-10°F. (23-9°C.)	74-87°F. (23-8°C.)	75-38°F. (24-1°C.)	74-56°F. (23-6°C.)	74-11°F. (23-4°C.)
	..	V. T.	0-929 inch	0-915 inch	0-941 inch	0-913 inch	0-908 inch
	..	R. H.	87-30 per cent	92-67 per cent	88-90 per cent	92-17 per cent	93-47 per cent
October	..	Max.	84-17°F. (28-9°C.)	85-60°F. (29-7°C.)	87-48°F. (30-8°C.)	84-09°F. (28-9°C.)	85-87°F. (29-9°C.)
	..	Min.	67-53°F. (19-7°C.)	70-36°F. (21-3°C.)	70-01°F. (21-1°C.)	68-80°F. (20-4°C.)	68-76°F. (20-4°C.)
	..	V. T.	0-748 inch	0-814 inch	0-843 inch	0-798 inch	0-800 inch
	..	R. H.	92-19 per cent	92-42 per cent	91-71 per cent	94-32 per cent	96-54 per cent
November	..	Max.	80-03°F. (26-7°C.)	78-29°F. (25-7°C.)	77-19°F. (25-1°C.)	81-17°F. (27-3°C.)	78-10°F. (25-6°C.)
	..	Min.	61-14°F. (16-2°C.)	56-36°F. (13-5°C.)	56-33°F. (13-5°C.)	57-50°F. (14-2°C.)	59-23°F. (15-1°C.)
	..	V. T.	0-629 inch	0-560 inch	0-548 inch	0-592 inch	0-586 inch
	..	R. H.	95-07 per cent	94-80 per cent	97-40 per cent	96-26 per cent	95-73 per cent
December	..	Max.	73-59°F. (23-1°C.)	75-70°F. (24-3°C.)	75-48°F. (24-1°C.)	75-56°F. (24-2°C.)	73-45°F. (23-0°C.)
	..	Min.	51-37°F. (10-8°C.)	51-23°F. (10-7°C.)	49-54°F. (9-7°C.)	48-13°F. (8-9°C.)	53-03°F. (11-7°C.)
	..	V. T.	0-467 inch	0-472 inch	0-430 inch	0-413 inch	0-474 inch
	..	R. H.	97-61 per cent	97-65 per cent	98-16 per cent	98-52 per cent	98-55 per cent



TABLE II.  
Rainfall in inches at Tocklai, Upper Assam, from 1918 to 1941.

Year.	Jan.	Feb.	Mar.	April.	May.	June.	July.	Aug.	Sept.	Oct.	Nov.	Dec.	Total rainfall.
1918	0.12	1.26	6.27	5.91	15.42	14.92	18.20	19.48	12.44	2.42	0.40	0.00	96.84
1919	0.28	0.97	0.75	7.41	6.57	22.09	16.37	10.71	9.59	9.40	0.42	0.11	84.67
1920	0.63	2.73	6.15	9.08	6.73	9.89	14.18	9.22	6.17	2.47	0.20	0.13	67.58
1921	1.63	0.86	4.21	14.03	9.28	7.71	14.52	9.71	14.23	5.15	0.03	1.14	82.50
1922	0.74	0.12	3.14	7.79	6.16	11.67	12.87	19.23	7.32	2.65	1.17	1.03	73.89
1923	0.00	2.77	0.43	10.84	11.97	20.19	18.79	17.11	14.12	3.93	1.55	0.11	101.81
1924	0.51	1.45	3.51	9.18	9.35	14.43	15.82	14.24	10.64	4.39	0.65	0.00	84.17
1925	3.15	0.80	3.56	7.03	15.11	9.50	21.36	3.79	10.79	4.33	0.83	0.00	80.25
1926	0.81	0.73	6.72	2.10	8.56	9.73	19.13	14.35	6.72	5.54	0.92	0.37	75.68
1927	1.40	3.44	3.85	9.78	4.64	17.12	13.85	15.66	16.90	5.87	0.74	0.00	93.25
1928	0.18	0.57	1.51	4.38	8.35	19.63	10.32	11.71	16.29	7.43	1.65	0.00	82.02
1929	2.82	0.03	3.23	11.72	14.20	18.84	17.84	11.48	9.32	4.66	0.21	0.92	95.27
1930	0.70	1.70	3.62	5.40	5.09	9.66	7.04	23.41	8.12	3.81	3.61	0.69	72.85
1931	0.64	0.92	0.70	11.78	6.66	15.64	13.54	13.33	9.79	4.56	0.79	0.75	79.10
1932	1.16	1.89	3.01	5.96	12.34	17.85	15.92	16.24	10.43	2.03	3.11	1.08	91.02
1933	0.24	1.48	0.52	9.68	4.34	10.96	12.87	18.72	9.33	4.11	0.25	0.22	72.72
1934	0.36	1.01	0.12	8.45	17.78	18.39	11.46	13.71	15.46	5.84	3.22	0.48	96.28
1935	0.11	2.08	3.48	4.42	10.79	18.51	17.13	12.21	12.63	0.15	0.37	0.03	81.91
1936	0.87	1.51	1.15	6.84	12.93	10.00	17.83	14.16	6.74	3.72	2.02	0.65	78.42
1937	0.13	3.10	1.05	2.61	10.16	10.46	16.77	12.21	10.58	4.80	0.24	0.12	72.23
1938	1.38	0.94	7.04	11.07	5.31	11.30	15.66	13.77	6.28	3.97	2.93	0.09	79.74
1939	0.07	1.31	0.70	6.51	8.30	12.99	10.89	9.11	8.49	4.33	0.00	0.00	62.70
1940	0.15	2.02	5.42	1.55	13.04	10.33	13.02	7.74	12.00	3.11	1.55	0.31	70.24
1941	0.06	1.56	1.26	12.57	4.75	14.56	10.27	15.47	..	..	..	..	..

all the adults which were used in experiments during a three-year investigation\*. Under the author's supervision, as many as 157 female *A. minimus* have been

\* The existence of such 'mosquito houses' is a point which assumes some importance in view of recent work by Viswanathan (1941) on the control of *A. minimus* by the spray-killing of adult mosquitoes.



taken in a single catch from one of these houses, but even higher catches of 300 or more have been recorded by a trained collector in an uncontrolled garden in Doom Dooma District.

### CLIMATIC CONDITIONS INSIDE COOLIE HOUSES.

From the meteorological figures shown in Table I, it is evident that there is a considerable range of temperature throughout the year, and that there must obviously be corresponding changes in the microclimate inside native houses and coolie huts. As the adult *A. minimus* spends such a large part of its life inside houses, it is necessary to have some idea of the range of conditions to which it is exposed in its resting places and feeding sites. It is also useful to compare conditions in a coolie hut with those in a typical cattle-shed, and to see if the differences observed can play any part in guiding this mosquito to suitable feeding or resting places.

*Rainy season.* During the months of July and August, when the conditions of temperature and humidity remain fairly uniform, continuous records of these two factors were made for a few weeks by means of thermo-hygrographs inside a standard Stevenson screen and inside a typical coolie house nearby, with thatch roof and mud plastered walls. The temperature records are summarized in Table III.

TABLE III.

*Shade temperatures compared with those inside a coolie house during the months July to September.*

STEVENSON SCREEN.			COOLIE HOUSE.		
Date.	Temperature.		Date.	Temperature.	
	Mean maximum.	Mean minimum.		Mean maximum.	Mean minimum.
10.vii-17.vii	32.8°C.	25.0°C.			
24.vii-31.vii	32.0°C.	25.0°C.			
31.vii-7.viii	32.3°C.	25.1°C.	31.vii-7.viii	30.1°C.	27.3°C.
			27.viii-3.ix	30.8°C.	27.3°C.

During this period the mean maximum temperature inside the coolie hut was 30.5°C. (86.9°F.), while that of the screen was 32.4°C. (90.3°F.).

The mean minimum temperature of the coolie hut was 27.3°C. (81.1°F.), compared with 25.0°C. (77.0°F.) for the screen. The mean maximum temperature of coolie huts is, therefore, about 2°C. lower, and the mean minimum about 2°C. higher, than the corresponding screen temperatures. During the hottest spells in Upper Assam, the shade temperature seldom exceeds 36°C. (97.5°F.), and this means that the highest temperature reached inside a thatched coolie



house is about 34°C. (93.2°F.). Temperatures inside houses with corrugated iron roofs are probably still higher, and a temperature of 34°C. has been noted at midday inside such a house where *A. minimus* was actually found resting. In the Dooars of North Bengal, shade temperatures of over 37.8°C. (100°F.) are occasionally recorded during the hot weather before the rains, so that 36°C. (96.8°F.) is about the highest temperature likely to be encountered inside a thatched coolie house. Normally, of course, the maximum temperature is considerably below this point.

Complete humidity records were made during one week of the rains, between 31.vii and 7.viii. The mean maximum relative humidity in the screen and in the coolie house was much the same, i.e., 92 per cent, but the mean minimum relative humidity in coolie houses was 78 per cent compared with 67 per cent recorded in the screen during the same period. During the period 28.viii to 3.ix the mean maximum relative humidity inside the coolie house was 91 per cent, whilst the mean minimum was slightly increased to 82 per cent.

*Cold weather.* Similar, but more detailed, observations were made in the cold weather, between the middle of October and the middle of February 1938–39. Continuous records of temperature inside two thatched coolie houses were made and compared with parallel records in the standard Stevenson screen outside. The two huts were chosen because they were of the type usually favoured by *A. minimus* as a resting place. The thermograph was placed on the floor under the bed, a common resting place of adults of this species, and an unheated room was selected. The conclusions are as follows:—

(1) At 6 p.m., approximately sundown, the temperatures inside coolie houses were from 2.0 to 3.6°C. higher than in the screen outside, the mean difference being 2.6°C.

At 6 a.m., approximately sunrise, the temperatures inside coolie huts were from 2.2 to 4.0°C. higher than outside, the mean difference being 3.0°C. These differences were maintained throughout the night between sunset and sunrise.

(2) The mean maximum temperature inside coolie houses was from 2.6 to 3.1°C. lower than the mean maximum screen temperature outside, the mean difference being 3.0°C.

The mean minimum temperatures inside coolie houses were from 3.1 to 3.8°C. higher than the mean minimum screen temperature outside, the mean difference being 3.3°C.

(3) During the coolest months, the lowest mean minimum temperature recorded inside the coolie houses for any week was 11.5°C. The lowest mean minimum temperature outside was 8.0°C.

During the cold weather, the relative humidity is at or near 100 per cent during most of the night, and the thick early morning mists sometimes hang about till 10 or 11 in the morning. Under such conditions, the hygrograph could not be expected to give accurate results, and therefore no humidity records were made.

About the lowest humidity conditions to which *A. minimus* is likely to be exposed are probably those which occur in some of the dry zones of the Dooars in March and April, where the relative humidity inside a coolie house may fall from 70 or 75 per cent, in the early morning, to 50 per cent or even lower in the hot hours of the afternoon. In the Assam Valley, however, the minimum



relative humidity inside coolie houses, even in the driest time of the year, seldom falls below 60 per cent, nor the maximum humidity below 90 per cent.

### TEMPERATURE CONDITIONS IN COWSHEDS.

It has frequently been assumed, without much justification, that animal houses, cow houses, stables and byres are warmer than human habitations at night, and that this temperature difference may account for differences in blood preference. This may well be the case where the cattle and human beings sleep in similar kinds of houses. In Assam, however, very few cow houses are built as solidly as coolie houses, most of them being large, light and airy owing to the fact that the bamboo walls seldom have mud or cowdung superimposed. A typical cow house of this kind with thatch roof was selected for temperature measurements. The house was empty by day, but contained about a dozen cows at night. Continuous temperature records were carried out as before inside the cow house and inside the Stevenson screen outside. The conclusions were as follows:—There is no appreciable difference between the maximum temperature attained inside the cow house and that of the screen: during two weeks the mean maximum differing by  $0.2^{\circ}\text{C}$ . in each case. During the night, however, the cowshed is constantly a few degrees warmer than the screen conditions, the mean minimum during two weeks differing by  $1.7^{\circ}\text{C}$ . and  $2.6^{\circ}\text{C}$ . respectively.

When we compare cowshed temperatures with those of coolie houses at the same time, the temperatures at night follow almost identical courses, the mean minima during the week differing by only  $0.6^{\circ}\text{C}$ . By day the cowshed temperatures always rise more sharply than those of coolie houses, and for one week the mean maximum of a cowshed was  $2.0^{\circ}\text{C}$ . higher than that of a coolie hut under similar conditions.

In trying to estimate the effect of climate on an insect, it is desirable to measure the conditions in the immediate environment of the insect. But in the case of *A. minimus* it is doubtful if this precision with regard to temperature and humidity is likely to be of much assistance. It is certainly true that the daytime resting places are usually about  $2^{\circ}\text{C}$ . cooler by day than the screen temperature outside, but this difference is so small that it seems unlikely to be of any real significance, except possibly under conditions of abnormally high temperature. Furthermore, very little is known about the nocturnal activity of *A. minimus*, and it is impossible to say what proportion of the night is spent outside and what proportion inside houses. The small but fairly constant differences of temperature inside and outside the coolie house might be supposed to play a part in attracting hungry mosquitoes to human habitations, but before discussing this possibility further it is necessary to know how mosquitoes react when faced with a choice of temperatures, and this behaviour is described in a separate section.

### LIGHT INTENSITY IN RESTING PLACES.

From the distribution of *A. minimus* inside houses it is apparent that light intensity is a most important factor in the choice of resting place, and mosquitoes are seldom found in situations where they can be seen with the unaided eye. In Assam, the majority of the cow houses are much lighter than coolie houses because the bamboo walls, when present, seldom have mud plaster superimposed, and in such sheds *A. minimus* is never found resting by day.



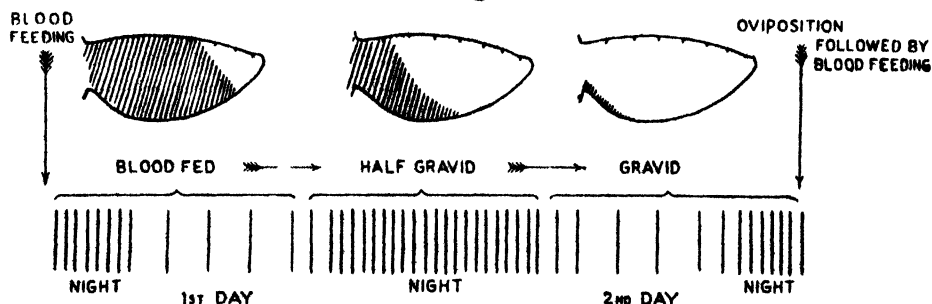
In attempting to measure the light intensity of resting places it is difficult to decide exactly how and when to measure it. The ideal would be to make the measurements at the actual time when adults are looking for a resting place, but, unfortunately, this presupposes a knowledge of the insect's nocturnal or early morning activities, which so far is not available. There are also considerable alterations in light intensity inside a house depending on the time of day, the degree of sunlight, and whether the door of the house is open or closed. Furthermore, if an attempt is made to measure the light intensity relative to that in the open outside, i.e., the light factor, there are few instruments available which can accurately cover such a wide range of light intensity.

Using a low illumination visual photometer (Thomson, 1940), a few readings were made underneath large bamboo beds where *A. minimus* was regularly found. Readings varied considerably according to the position of the photometer, but one conclusion which seemed fairly certain was that even on a bright cloudless day, about 11 a.m., the light intensity of ideal resting places was usually less than 0.01 foot candle.

### DIGESTION OF BLOOD AND MATURATION OF OVARIES.

Before discussing the population of mosquitoes and their behaviour in houses, it is necessary to know how long it takes the female to digest its blood meal and to mature its ovaries. The length of the period between feeding and oviposition is influenced by climatic conditions, and we would expect to find in Assam wide differences between monsoon and the cold weather conditions. For four or five months the plains experience fairly uniform conditions of high temperature and high humidity (Table I) and during this period *A. minimus* normally takes two days to digest its blood meal. For example, if feeding takes place on a Monday night, the mosquito is ready to lay eggs by the following Wednesday night (Text-figure), one blood meal being sufficient for

Text-figure.



The course of digestion of the blood meal and maturation of the ovaries of *A. minimus* under monsoon conditions, June to October, and the terms used in the paper to describe these stages.

the maturation of the ovaries. Even under the highest temperature conditions experienced in Assam this is the minimum duration. Under such favourable conditions practically all the adults caught in daytime resting places can be divided into three categories:—

- (1) *Blood fed*. Those mosquitoes which fed during the previous night and in which blood occupies most of the abdomen.



- (2) *Gravid*. Those adults which fed two nights ago and in which the abdomen is mostly occupied with ovaries, with blood either completely digested or confined to a narrow ventral strip.

For example, if the collection was made on Monday forenoon, those of lot (2) would oviposit on Monday night, while those of lot (1) would be ready to do so on Tuesday night.

- (3) *Unfed*. This category contains the occasional mosquito which has failed to obtain a blood feed overnight.

Considering the favourable climatic conditions, therefore, *A. minimus* takes rather a long time to maturate its ovaries, and it compares unfavourably with a species such as *A. vagus*, which, under similar conditions, digests its blood in half the time: females of *A. vagus* which feed on Monday night have digested most of their blood meal by Tuesday morning and are ready to oviposit on Tuesday night, one night after feeding.

From October onwards there is a steady fall in temperature reaching a minimum about the end of December and the beginning of January (Table I). Under these conditions, digestion of blood takes place more slowly and there is a longer period between feeding and oviposition. However, even the lowest temperatures experienced in the plains of Upper Assam neither prevent the maturation of ovaries nor induce any state of hibernation. Throughout the coldest months, freshly blood fed specimens of *A. minimus* can be found regularly inside coolie houses, and in nearly every case oviposition follows within four to six days after feeding.

Blood fed females of *A. minimus* caught in the latter part of December were kept in an insectary where the temperature conditions were very similar to those inside coolie houses, with a mean maximum temperature of 19.6°C. (67.3°F.) and a mean minimum of 10.5°C. (51°F.), and a relative humidity from 80 to 100 per cent. Under such conditions, digestion of the blood and maturation of the ovaries took three times longer than under monsoon conditions, most of the eggs being laid six nights after feeding.

These observations, together with the fact that freshly blood fed females are regularly found throughout the coldest part of the year, confirm those of Rice and Mohan (1936) in showing that feeding, digestion of blood, and maturation of ovaries followed by oviposition continue right through the cold weather, and that there is no hibernation of females of *A. minimus* in the plains of Assam.

#### COMPOSITION AND SIZE OF POPULATION IN HOUSES.

In the previous section it was shown that during the monsoon months nearly all specimens of *A. minimus* caught by day can be divided into two distinct types, i.e., *blood fed* and *gravid* (Text-figure). In nearly every catch the number of blood fed females greatly exceeds the number of gravids: for example, out of 1,338 females caught in the same two or three coolie houses in July and August, 871, or 65 per cent, were blood fed. In catches for one day the proportion of blood fed mosquitoes was sometimes as high as 78 per cent, and it was only occasionally that the number of gravid females exceeded that of the blood fed.



This division into two stages is convenient, but it gives little indication of the age of the mosquitoes. Without more detailed work, it is impossible to say what proportion of the blood fed mosquitoes are recently emerged, and whether the gravid females are on the average older than the blood fed ones or not. But from the regularity with which the number of blood fed mosquitoes exceeds the number of gravids, it seems probable that this disparity affords some indication of the daily mortality under natural conditions.

If attention is concentrated on a few selected houses and catches are regularly made, it soon becomes evident that there must be a considerable turnover of mosquitoes, and that collecting all mosquitoes from the same houses two or three times a week has little effect on the general population. Even when the mosquitoes are collected from the same hut on consecutive days, there is no drastic change in the total population. For example, two expert catchers collected 118 blood fed and 43 gravid *A. minimus* from two huts one morning; on the following morning collections in the same huts yielded 85 blood fed and 41 gravid. In a second experiment, the total collection of mosquitoes from three huts amounted to 35 blood fed and 27 gravid females; on the following day the same huts yielded 49 blood fed and 14 gravid. In a third experiment, three huts yielded 37 blood fed and 62 gravid females; on the following day 68 blood fed and 20 gravid females were collected from the same huts.

From these figures it is evident that most of the population in a coolie hut is replaced each day, not only by new mosquitoes coming in for a blood meal but also by gravid females. The presence of gravid females on the second day of catching shows that during the period between feeding and oviposition there must be a certain amount of migration between houses; most of the gravid females in the second day's catch must have come in from elsewhere, as very few mosquitoes could have escaped the careful search of the previous day.

In a group of houses equally exposed to visits from *A. minimus*, the largest number of mosquitoes were found resting by day in those houses which offered suitable resting places as well as opportunities for feeding. This may sound elementary, but, on the other hand, there is no definite relation between the number of *A. minimus* feeding in a house at night and the number of blood fed females resting in that house on the following day. For example, large numbers of *A. minimus* were found in a particular house every time it was searched. One day the large bamboo bed, which occupied half of the darker room and was the ideal resting place for mosquitoes, was removed; from that time onwards very few specimens of *A. minimus* were found in that house compared with contiguous houses, although there is no reason to suppose that feeding activities by night had appreciably diminished.

Unoccupied houses may also yield a small number of *A. minimus*, provided they offer suitable daytime resting places, and a few freshly blood fed as well as gravid females may be found in such places. In general, however, empty houses play only a very minor part as daytime resting sites.

Before going on to further discussion of the nocturnal movements of *A. minimus* and the choice of a resting place, it will be convenient to describe the reactions to light at sunset and sunrise, as the behaviour at that time does much to explain the general activities discussed.



REACTIONS OF *A. MINIMUS* TO LIGHT AT SUNRISE AND SUNSET.

Our observations in the previous sections have shown that there is a considerable daily turnover in the population of *A. minimus* inside houses, and that many mosquitoes migrate between houses during the period between feeding and ovipositing. Adults disturbed in their daytime resting place show no desire to leave the house, but fly around slowly and eventually settle in another dark place. It seems fairly certain that once they have found a suitable resting place, presumably early in the morning, they tend to remain there throughout the day.

It is commonly believed that in the early part of the evening mosquitoes are driven out of the houses by the smoke of cooking fires, and it is certainly the case that for an hour or two after sundown very few specimens of *A. minimus* can be found inside houses compared with daytime catches. But the smoke of fires cannot be responsible for this behaviour, because during the day many specimens of *A. minimus* have been found resting in rooms filled with smoke. We must look elsewhere for an explanation of this exodus from houses, and the laboratory observations are particularly helpful in explaining these reactions.

*A. minimus* adults were bred out in the laboratory and kept in large mosquito-netting cages,  $2 \times 2 \times 2$  feet. During the day, the mosquitoes rested quietly in the cage, even though the insectary was well illuminated. Towards sundown, however, they became very active and were all attracted towards the window while there was still light outside. The darker end of the cage was deserted, both males and females crowding to the other end. Similar activity at sundown has been described for *A. gambiae* by de Meillon (1937), but *A. minimus* not only shows a strong photokinetic reaction, but also a real phototaxis, or attraction towards light. The first observations were made on males and unfed females, but precisely similar behaviour was shown by freshly gorged females. The period of greatest attraction to light lasts for about half an hour: it starts very suddenly about a quarter of an hour before sunset, when the intensity of illumination in the open is changing rapidly, and ends at a time when it is just possible to read print outside. After that time all activity in the cages soon ceases.

This behaviour is evident over a wide range of temperature and humidity conditions, and furthermore, during the period of greatest activity the changes in these two factors are so slight that they can have little influence in initiating the sudden activity. The rapid change in light intensity at that time is probably the principal factor involved, but the behaviour might also be influenced by some sort of diurnal rhythm.

As all the *A. minimus* bred out in the laboratory, whether blood fed or hungry, were unfertilized, as will be described later, it was decided to repeat these experiments more carefully with wild-caught females, and under conditions more closely resembling those in nature.

In one side of the  $2 \times 2 \times 2$  feet cage 1 square foot of netting was removed, and over the opening a small cage 1 foot cube was fixed. The outside of the large cage was covered with black cloth, and a curtain of black cloth could be drawn across the opening into the small cage. The small cage directed towards the insectary window was left uncovered. The whole apparatus was meant to resemble a dark coolie hut, with light coming in from outside through small windows or gaps in the wall.



In the first experiment, 70 blood fed specimens of *A. minimus* caught inside coolie huts by day were put in the large cage in the afternoon, the opening between it and the small light cage being closed. When the mosquitoes had settled down in the large cage, the communicating curtain was opened to admit a small beam of daylight; the mosquitoes remained quiet and none flew into the small light cage. The cage was examined at about 6 p.m., a little after sunset, and it was found that the great majority of mosquitoes had left the dark cage and were actively attracted to light in the small cage. At the height of activity the communicating curtain was closed and 3 females were found still resting in the dark cage, i.e., about 96 per cent of the mosquitoes were attracted to light at sundown, even from a dark daytime resting place.

In a second experiment with 50 blood fed females, about 88 per cent were attracted to light at sundown, 6 mosquitoes still remaining in their resting place. When the activity gradually came to an end about half an hour after sunset, all the mosquitoes which had been attracted into the small light cage remained there, none returning to the dark resting place.

Those females which were recorded as blood fed during the day would, according to the simple convention adopted in this paper, be classified as 'half gravid' at night (Text-figure) and would not be ready to oviposit for a further 24 hours. The exodus of gravid females from the house at sundown is understandable as they are ready to oviposit, but there seems no reason why the half gravid females should do so instead of remaining in the same resting place overnight. Nevertheless, the attraction to light at sundown is so well marked in all these experiments that there can be little doubt that this is what actually happens under natural conditions, probably about 8 or 9 out of every 10 half gravid females leaving the shelter of the coolie house at dusk.

On the following day, these half gravid females will be listed as gravid found resting inside houses (Text-figure). What happens to these mosquitoes from the time they leave the house at sundown till the following morning when they are again found inside houses, is quite unknown. It is interesting to note that not *all* the mosquitoes leave the house at dusk, a small proportion evidently remaining in the daytime resting place overnight. This has been confirmed by the capture of half gravid females in dark corners of the house shortly after dark.

It remained to be seen whether there was a similar reaction at dawn. It has already been pointed out that *A. minimus* is seldom found in a well illuminated room or house by day, even though it is almost certain that feeding takes place there at night. This was confirmed by more exact observations described later, and it is, therefore, certain that females which feed inside such a house at night will leave it in the morning if there are no suitable daytime resting places. But, in order to see if there was any general attraction to light at dawn, the laboratory experiments were repeated in the early morning. Fifty blood fed females caught in coolie houses were put in the large dark cage in the afternoon, the entrance to the small cage being closed. An hour or two after sunset, when all activity had died down, the communicating curtain was opened and no mosquitoes were found in the light cage. The cage was watched between 5 and 6 a.m. on the following morning. At dawn there was no general activity or attraction to light; and by the time there was enough light outside only 4 females had left the dark cage and entered



the small light cage. In a second experiment, 7 out of 65 blood fed females were attracted into the light cage at dawn.

It appears, therefore, that the great activity of all stages at sundown is not repeated at dawn, although a small proportion of 1 or 2 in 10 may leave the house in the early morning even when suitable daytime resting places are available.

### MARKING EXPERIMENTS.

In the coolie lines of one tea garden it was found that a small group of houses at one end of the line was particularly frequented by *A. minimus*, large numbers of adults being regularly captured in two or three of these houses. This seemed to afford a good opportunity for studying the movements of mosquitoes between houses, and a series of marking experiments was therefore carried out. As only small numbers of *A. minimus* were available at one time, the method of marking which was selected as being most suitable was that of Majid (1937), using fine powders such as printers' 'gold' and 'silver'. Mosquitoes caught inside each house were lightly anaesthetized, a record was made of their condition whether blood fed or gravid, and some fine powder was lightly dabbed on the ventral surface of the body ensuring that the fine grains were firmly attached. As the mosquitoes recovered they flew back into the dark corners of the same room in which they had been captured; careful watch was kept but none was seen to leave the house. On the following day, all mosquitoes were collected from the same house and a record was made of the marked mosquitoes captured, their condition and where they were found. The collections were confined to the same group of four or five houses, as the careful search of many more houses would have required a large staff of trained collectors.

All marking experiments were carried out in three huts which stood in a straight line about 20 yards from each other. As the breeding places were very diffuse, it is impossible to state the source of most of the adults, but small earth wells in among the coolie lines provided minor breeding places. The huts in which mosquitoes were marked and released were called huts (1), (2) and (3).

*First experiment:* On October 17, 37 gravid females caught in hut (1) were marked with gold powder and liberated in the same dark room in which they were caught. On the following day, 4 marked females were recaptured, all being now blood fed. All were conspicuously marked with numerous gold flakes, and could be distinguished by the naked eye; 3 of them were caught in hut (1) and 1 in hut (2). This shows beyond doubt that gravid females oviposit and return for a blood meal the same night. The fact that 3 of the 4 recaptured mosquitoes were found in the same house in which they had been liberated is also interesting. No collections were made on October 19, but on the 20th 2 marked mosquitoes were recovered, 1 in hut (1) and 1 in hut (2); both being blood fed.

*Second experiment:* On October 25, 13 blood fed females found in hut (3) were marked with gold and liberated in the same house. On the following day search in the three huts produced one marked gravid female in hut (3).

*Third experiment:* On November 2, 31 blood fed mosquitoes were caught in hut (2), marked with gold and liberated in the same house. On the following



day, 58 *A. minimus* were caught in the three huts but none of them was marked.

*Fourth experiment:* On November 10, 26 blood fed females caught in hut (1) were marked with gold and liberated in the same house. On the following day, search in five huts yielded 5 marked mosquitoes, 4 of them in hut (1) and 1 in hut (3). Of the 5, 4 still contained half-digested blood and 1 was gravid. At this cool time of the year, the digestion of the blood meal takes three days or more, as compared with two days under monsoon conditions.

*Fifth experiment:* On July 5, all the mosquitoes in coolie huts (2) and (3) were collected, those from hut (2) being marked with printers' 'silver' powder and those from hut (3) with 'gold'. Marked mosquitoes were liberated in the same house in which they had been captured. On the following day the two huts were carefully searched again. The results are shown below:

Hut.	Marked on 5.vii with silver.	Total collection on 6.vii.	Marked specimens recovered.
(2)	Blood fed .. 79	Blood fed 64	1 Blood fed 'gold'.
	Gravid .. 22	Gravid 26	2 Gravid 'silver'.
(3)	Marked on 5.vii with gold.		
	Blood fed .. 39	Blood fed 21	
	Gravid .. 21	Gravid 15	3 Gravid 'gold'.

This experiment gives a good idea of how the mosquito population in a coolie hut is replaced each day, as shown by the large number of unmarked gravid females, which must have come in from other houses. Also, out of 118 blood fed females caught and marked in the two huts on the first day, only 5 were recovered as gravid females on the second day, all these 5 being found in the same houses in which they had been liberated; also, the complete absence from hut (2) of gravid females marked with gold, and from hut (3) of gravid females marked with silver suggests that the nocturnal migrations of blood fed females—which by that time have reached a half-gravid condition (Text-figure)—take them further afield than the next door house. Of the 43 gravid females marked on 5.vii, all would probably leave the house to oviposit that night, and of these, one from hut (3) returned later in the night for a blood meal in hut (2).

All these marking experiments confirm the previous observations regarding the daily turnover and the exodus of most mosquitoes from the house at sundown. They also show a surprisingly large number of marked mosquitoes recovered, 18 out of a total 268 marked specimens of *A. minimus* being recaptured. When we consider that search for marked mosquitoes was almost entirely confined to the same three huts and that collections were not carried out every day, the conclusion seems to be that the nocturnal migrations of *A. minimus* in that particular coolie line take place on a fairly small scale and within a comparatively small radius. Under such conditions, a female may return to the same house or group of houses for blood feeds two or three times in its life. The method of investigation is decidedly promising, and the amount of interesting and valuable information which can result from this technique applied on a much larger and more detailed scale



is well shown by the observations on *A. culicifacies* recorded by Afridi *et al.* (1940).

#### NOCTURNAL ACTIVITY AND TIME OF FEEDING.

In the tea garden coolie lines in which all the previous catches and experiments were made, conditions seemed ideal for observations regarding the nocturnal activity of *A. minimus*. Since these houses regularly contained high populations of *A. minimus*, catches at different times of the night would have yielded valuable information. However, there were difficulties in the way of interfering with the occupants of the houses at night time, and observations under ideal conditions were not carried out till later, in another district.

The first series of observations was, therefore, made in the garden hospital, which was light and airy, with no suitable daytime resting places for *A. minimus*.

*First experiment:* On 7.vii, the first collection in the hospital was made by the author and one catcher from 10 to 10-30 p.m. The majority of the patients were sleeping outside on the verandahs round the hospital buildings, and only two people were sleeping inside. At 10-30 p.m., only a few specimens of *A. minimus* were found resting outside and inside the walls of the houses: only 5 were caught, and all were half-gravid, i.e., they had fed the previous night (Text-figure). The second catch was made before dawn between 4-30 and 5 a.m. Thirty-one blood fed females were taken, nearly all resting on the outside walls, beside the sleeping patients: a few were found inside the hospital and all mosquitoes were fully gorged. The results were—

*First collection, 10 to 10-30 p.m.*

Blood fed	..	0
Half-gravid	..	5

*Second collection, 4-30 to 5 a.m.*

Blood fed	..	31
Gravid	..	0

*Second experiment:* On 21.vii, the above experiment was repeated in exactly the same way with patients sleeping both inside and outside. The results were—

*First collection, 10 to 10-30 p.m.*

Blood fed	..	0
Half-gravid	..	5
Unfed	..	2

*Second collection, 4-30 to 5 a.m.*

Blood fed		51
Gravid	..	2

In both these experiments, there was a complete absence of blood fed specimens of *A. minimus* in the first catch, and relatively large numbers of blood fed specimens in the second catch indicating that most of the blood feeding took place later than 10-30 p.m. The occurrence of half-gravid specimens both inside and outside is in accordance with previous conclusions regarding the nocturnal movements of that stage. Both observations also show that *A. minimus* can not only feed outside houses but may remain outside till dawn after feeding. Freshly blood fed specimens of *A. minimus* were seen resting on the outside walls up to



the time when there was light enough to distinguish the presence or absence of suitable, dark, daytime resting places inside a house. These catches also show that *A. minimus* may be completely absent from a well-illuminated house by day, even though considerable blood feeding actively takes place there at night.

In the following year, similar observations were carried out in Doom Dooma District, where the surveyor had regularly recorded large catches of *A. minimus* in the coolie lines of an uncontrolled garden. All the coolie huts were dark, with abundant daytime resting places. The first collection was made later in the night than before, between 11 p.m. and midnight, by which time all the occupants had been sleeping for several hours. The second collection was carried out next morning about 9 to 10 a.m.

*Third experiment:* Three coolie huts were searched from 11 p.m. to midnight and the same three huts searched again next morning.

*First collection, 11 p.m. to 12 midnight.*

Blood fed	..	8
Half-gravid	..	54
Unfed	..	5

*Second collection, 9 to 10 a.m.*

Blood fed	..	98
Half-gravid	..	32
Unfed	..	1

*Fourth experiment:* This was carried out exactly as above in three other coolie huts.

*First collection, 11 p.m. to 12 midnight.*

Blood fed	..	9
Half-gravid	..	42
Unfed	..	2

*Second collection, 9 to 10 a.m.*

Blood fed	..	52
Half-gravid	..	38
Unfed	..	0

The combined results show that 17 blood fed females were caught in the first collection, while 150 were caught in the second collection, i.e., approximately 90 per cent of the blood feeding took place in the latter part of the night, from midnight to dawn. In the first collection, many half-gravid adults were taken in the usual daytime resting places, suggesting that they had not left the house at sundown. The second collection shows that a large number of half-gravid or gravid females must enter the house after midnight, between the first and second collection and presumably at dawn.

Although all these nocturnal catches were carried out on only four nights, they were made under perfectly natural conditions, and from the consistency of the figures they almost certainly represent the usual behaviour at night during the monsoon. The most important implication from these observations seems to be that the chances of being bitten by *A. minimus* are considerably greater in the latter part of the night, or even just before dawn, than they are at dusk and the first two or three hours after dark. This conclusion is not altogether surprising, because we have already seen that females, after ovipositing at night, return for a blood meal the same night, and such females, including infective ones, would naturally tend to feed rather later than the first hour or two after



sundown. The fact that feeding may take place right up till dawn has been confirmed by direct observation on sleeping hospital patients.

#### NUMBER OF EGGS LAID AT DIFFERENT SEASONS.

Previous observations had shown that *A. minimus* usually oviposits readily in the laboratory, and gorged females caught inside coolie houses were accordingly each put in a separate cage with a dish of water and allowed to lay their eggs. Only those females were considered which remained alive and active after laying all their eggs. The results are shown in Table IV.

TABLE IV.

Month.	Number of <i>A. minimus</i> .	EGGS PER FEMALE.		
		Maximum.	Minimum.	Mean.
July ..	8	161	78	139
August ..	12	175	97	135
September ..				
January ..	9	104	53	82

} 137

Although the number of mosquitoes used in this experiment is rather small to give an accurate idea of egg laying at different seasons, it would appear that the average number of eggs laid in July, August and September, when temperature and humidity are constantly high (Table I), is much greater than that of January, which is about the coldest month in the year. Twenty egg counts made by Rice and Mohan (*loc. cit.*) between January 15 and February 15 showed a mean of 97, with a range of 81 to 118. Those figures, while slightly higher than the figures for January recorded above, are still well below those of the monsoon months. Whatever the reason, therefore, it seems fairly certain that in the coldest months *A. minimus* only lays about two-thirds the number of eggs that it does in the monsoon season.

#### MATING AND FERTILIZATION.

When this investigation on *A. minimus* was started, it was confidently hoped to establish a colony of this species in the laboratory, and possibly a self-perpetuating one. Despite repeated attempts, this was a complete failure; and as far as the author is aware, no one else has succeeded in doing so. The main obstacle was the refusal of adults to mate in captivity, whether in large cages or small cages, outside or inside buildings. In small cages measuring  $2 \times 2 \times 2$  feet, 2 or 3 males were occasionally seen making swarming movements at sundown, but no actual mating was seen and no fertilized females were ever found.

The *A. minimus* adults in these cages, which were all bred out from larvæ or pupæ, were regularly given the opportunity of feeding on the author's arm at night, a large number taking full blood meals on each occasion. In these unfertilized females the blood meal was soon digested, but there was no parallel development of the ovaries: the abdomen resumed its flat, starved condition, and



no eggs were ever laid in these cages. This behaviour is particularly interesting in view of the recent observations of Roy (1940) in which he showed that a blood meal failed to lead to egg formation in virgin females of *A. subpictus*, but did so in *A. annularis* and *A. stephensi*. The behaviour of *A. minimus* in this respect, therefore, resembles that of *A. subpictus*\*.

In such species, therefore, virgin females can take full blood meals several times without egg formation, but it would be entirely misleading to imagine that this is what happens under natural conditions. In the field such an abnormal event could never occur, and in the case of *A. minimus* (and also *A. vagus*) for certain, one blood meal is sufficient for complete egg formation in a fertilized female. When wild caught females have oviposited in the laboratory, a single blood meal is sufficient to bring about once more maturation of the ovaries and eventual oviposition. It is necessary to stress this point, because great confusion can be caused by overlooking the wide differences which may exist between ovarian development of virgin females in the laboratory and that of fertilized females under natural conditions, and also by assuming that all species of *Anopheles* react in the same way. Toumanoff (*loc. cit.*), encountering the same difficulty with laboratory bred specimens of *A. minimus*, *A. maculatus* and *A. vagus*, wrote that 'if one therefore agrees with Darling and Christophers and other authors that the ovaries may develop without the female being fertilized, one must conclude that the majority of species in Indo-China . . . need several blood meals to mature their eggs'. As this is a point of first rate epidemiological importance, it is necessary to emphasize the fallacy in Toumanoff's statement, and to correct the very erroneous impression which it gives of the behaviour of vector species in that part of the world.

#### FEEDING OF ANIMALS.

It has long been recognized that *A. minimus* is a particularly dangerous vector of malaria because of its marked preference for human blood; and its high androphilic index has been demonstrated by Ramsay *et al.* (1936) and by Toumanoff (*loc. cit.*). It was desirable to ascertain whether this high index is really due to a decided host preference or to some physical difficulty in piercing the skin of other animals. A few simple experiments were, therefore, carried out in order to see to what extent *A. minimus* can feed on animals in the absence of man. An empty coolie hut with the usual thatch roof and mud plastered bamboo walls was selected, and inside this was placed a large wooden pen or cage in which the animal could be confined at night. Over this was a large mosquito curtain which hung from the ceiling to the floor. The animal was put in the house about an hour before dusk and a batch of hungry *A. minimus* introduced through a sleeve in the net. On the following morning, all the mosquitoes inside the net were caught by two collectors and a count was made of the fed and unfed insects. All the *A. minimus* used in these experiments were wild caught females, which had recently laid their eggs in the laboratory. The figures which were considered of importance were: (a) the percentage of live mosquitoes recovered in the morning which were blood fed, and (b) the number of blood fed females recovered expressed as a percentage of the total number introduced the previous night. The two figures together could be used as a crude index for comparing the suitability of different animals

\* Complete failure by the present author to establish a colony of *A. vagus* for the same reason, shows that this species also comes in the same category.



as hosts. The number of mosquitoes introduced each night varied according to the number available, but the average was about one hundred. In the experiments the following animals were used:—1 calf, 1 goat, 2 fowls, and 1 dog. Difficulty was encountered in confining a dog in the cage overnight, particularly in the case of *pariah* dogs which broke their way out time and again. The figures available for dog, therefore, while showing that *A. minimus* will readily feed on that animal, cannot be compared with those for other animals which remained quiet at night. The results are shown in Table V.

TABLE V.  
*Feeding experiments.*

Animal.	Number of experiments.	Number of hungry <i>A. minimus</i> introduced.	Total recovered in morning.	Blood fed.	BLOOD FED EXPRESSED AS PERCENTAGE OF:	
					Mosquitoes introduced.	Mosquitoes recovered.
Cow ..	13	1,354	592	498	37	84
Goat ..	7	644	147	72	11	48
Two fowls .	6	436	125	46	11	37
Dog ..	3	182	66	53	29	80

The figures show that in the absence of the normal host, man, *A. minimus* can make use of all these different animals. At the same time, it appears that, as an alternative host, cow is much more suitable than either goat or fowls. Figures for dog are scanty for reasons described above, but they suggest that it might prove almost as suitable an alternative host as cow. To what extent this alternative choice is influenced by the bulk of the animal is difficult to say, but the ease with which *A. minimus* fed on a very small dog seems to discount the importance of size, and suggests that, quite apart from bulk, some animals are more suitable than others as alternative hosts to man.

In any case, one indisputable fact which emerges is that, in the absence of man, *A. minimus* can readily feed on animals, and that the high androphilic index must be due principally to a host preference, the exact significance of which remains a complete mystery.

A great deal has been written about animal deviation and the possible protective influence of animals near the house, and it is evidently a subject which lends itself admirably to theorizing and speculation. Although no attempt was made to follow up this question in greater detail, it might be interesting for future reference or for comparison with other countries to have some idea of the animals which live in association with man in Assam.

A census of people and animals was made in a typical tea garden coolie line in Upper Assam (imported labour) and in a typical Assamese *bustee* or village with an indigenous population. The results are shown in Table VI.

In the Assamese village, all the houses had animals nearby, while in the coolie lines 47 per cent of the houses had no animals of any kind. All the



TABLE VI.

*Census of men and animals in (A) Assamese village, and (B) coolie line.*

	Houses.	Men.	Cows.	Goats.	Ducks.	Fowls.	Pigeons.	Dogs.	Cats.	Total animals.
A.	26	177	115	48	466	0	60	38	37	764
B.	66	209	104	12	20	113	2	6	1	258

Assamese houses possessed cows, but only 30 per cent of the coolie huts. The proportion of human beings to cows in the coolie lines was 2 : 1, while in the village it was 3 : 2. In a few coolie huts, men, cows, and goats all slept under the same roof, but cows were usually kept in a separate shed beside the house, and this was always the case in the Assamese village.

From all these figures it appears that if animals afford any protection, the Assamese villager would be in a much better position than the garden coolie. Although we have no further relevant data in Assam, the results obtained by Toumanoff (*loc. cit.*) are particularly interesting in this respect. By making precipitin tests of the blood meals of *A. minimus* caught where domestic animals were present in different numbers, he was able to find the effect of different degrees of animal protection on the androphilic index of the mosquito. Increasing the number of cattle close to the house produced a corresponding decrease in the number of *A. minimus* feeding on man, but this deviation was only partial, and even under the most favourable conditions of animal protection 50 per cent of the mosquitoes still contained human blood.

#### FEEDING ACTIVITIES IN THE COLD WEATHER.

It has already been pointed out that freshly blood fed females of *A. minimus* are found throughout the cold weather, even in the coldest weeks of the year. In order to find out if there is any difference in the degree of feeding activity between the monsoon conditions and the cold weather conditions, some observations were carried out in the same animal house as was used in the experiment recorded in the previous section. In six experiments conducted between November 24 and December 18, 165 hungry *A. minimus* were given the chance of feeding on the calf. Of these, 69 were recovered, of which 52 were blood fed. The blood fed mosquitoes, therefore, make up 32 per cent of the total introduced and 75 per cent of those recovered. In the same hut, under monsoon conditions during July and August, the corresponding figures were 37 per cent and 84 per cent. In the cold weather experiments a continuous thermograph record was kept of night temperatures inside the animal house to show the actual conditions under which feeding took place: the maximum temperature at night was taken as that recorded at 6 p.m., while the minimum was usually about dawn between 6 and 7 a.m. The night temperature gradient fell from a maximum of 21.0°C. and a minimum of 14.5°C. on 24.xi to a maximum of 17.0°C. and a minimum of 10.5°C. on 18.xii. As shown in the section on climatic conditions, the lowest mean temperature recorded inside a coolie house for any week in the cold weather was 11.5°C.; these feeding experiments, therefore, embrace the whole range of temperature to which the feeding of *A. minimus* in houses is likely to be



exposed in the plains of Assam. From the proportion of blood fed females recovered in the experiments described above, there appears to be a slight fall in blood feeding activity in the coldest time of the year, but the differences are so small that there is certainly no indication of a drastic change of behaviour, or of any marked inhibitory effect of cold weather temperatures on blood feeding.

#### REACTIONS OF *A. MINIMUS* TO HUMIDITY.

*A. minimus* spends most of its adult life in conditions of high humidity, usually within a range 60 to 100 per cent, although in some dry areas in the Bengal Dooars the relative humidity inside houses may fall to 50 per cent or even lower during the hottest hours of the day. In order to find out if humidity is a factor which influences the behaviour of the mosquito between sundown and dawn, experiments were carried out in the laboratory to test the reactions of females to a choice of different relative humidities. The method of investigation was precisely the same as that used in studying the reactions of *Culex fatigans* (Thomson, 1938), females being introduced into a circular glass chamber in which a constant difference of relative humidity, from 5 to 50 per cent, could be maintained between the two halves of the dish by means of different salt solutions. The mosquitoes were offered the choice of 75 and 95 per cent R.H., 50 and 95 per cent R.H., etc., each experiment being carried out with females of uniform condition, such as, all blood fed, all newly emerged, all gravid, etc., as it was shown in the case of *C. fatigans* that such conditions influence the reactions to humidity. When exposed to different choices of relative humidity between 50 and 100 per cent on the humidity scale, the behaviour of *A. minimus* at all stages was quite erratic. There was never any marked humidity preference either by day, at sunset or at night, and it seems unlikely, therefore, that this factor plays any major part in influencing the choice of resting place or the movements in and out of houses.

The reactions to temperature, on the other hand, are very striking and will now be described.

#### REACTIONS OF *A. MINIMUS* TO TEMPERATURE.

The behaviour of *A. minimus* adults when faced with a choice of temperature was studied by means of the temperature gradient apparatus described fully in a previous paper (Thomson, 1938). The insects were introduced into a circular glass chamber, the opposite sides of which were partly immersed in different constant temperature water baths, so that a constant difference of temperature, such as 5°C. or 10°C., could be maintained between each half of the chamber. By this means it is possible to compare the sensitivity to temperature at different parts of the temperature scale. When a difference of temperature is set up between the two halves of the chamber, there will also be a gradient of relative humidity and saturation deficiency; but as we have already found that *A. minimus* does not react to differences in relative humidity between 50 per cent and 100 per cent, it will be sufficient to adjust the absolute humidity inside the closed temperature gradient apparatus in such a way that the extremes of humidity eventually set up will be well within the inactive zone, in which case the behaviour of the mosquitoes cannot be influenced by the humidity differences present. In the present series of



experiments, the range of humidity inside the apparatus was limited by means of shallow dishes containing a saturated solution of sodium chloride.

Each experiment was started in the morning, mosquitoes of a uniform condition being introduced into the chamber through a small hole in the glass roof, when a constant temperature difference had been set up between each half. The apparatus was immediately covered with black cloth, and after a standard period of 20 or 30 minutes, a count was made of the mosquitoes which had settled in each half of the chamber. The mosquitoes were then disturbed with a bent wire introduced through the opening in the roof, and when most of them were in flight the opening was closed and the black cloth quickly replaced. About 10 or 12 readings were taken in a similar way, and the figures were added up to give the total number which had settled on each side of the chamber.

*Blood fed females.* Freshly gorged females were introduced into this apparatus and exposed to a choice of 5°C.

Exposed to a choice of 20°C. to 25°C., 48 per cent of 151 females settled on the cool side, showing no marked preference.

Exposed to a choice of 25°C. to 30°C., 54 per cent of 224 females settled on the cooler side, again showing no marked preference.

Exposed to a choice of 30°C. to 35°C., 94 per cent of 209 females settled on the cooler side, and throughout the avoidance of the warmer side was very marked and consistent.

It appears, therefore, that above 30°C. blood fed females show a marked avoidance of higher temperatures, while below 30°C. the reaction disappears.

*Hungry females.* When exposed to a choice of 20°C. to 25°C., the behaviour of hungry unfed females was erratic and there was no preference for a particular side.

Exposed to a choice of 25°C. to 30°C., 96 per cent of 247 females settled on the cool side of the chamber, showing a regular avoidance of the higher temperature.

Below 20°C., there was no marked reaction in either blood fed or in hungry females, and never any noticeable attraction to the warmer side.

At all stages, including gravid females, the main feature of the behaviour was the avoidance of high temperatures, the response being distinct above 25°C. in hungry females, and above 30°C. in blood fed ones.

In the same apparatus, *C. fatigans* (Thomson, 1938) showed the same general behaviour, hungry females again showing a much stronger avoidance of higher temperatures than blood fed females. It is interesting to note that there is never any indication, even below 15°C., of hungry females being attracted to the warm side either during the day or at dusk. In a previous section, it was shown that during the night the air inside a thatch native house or coolie hut is always a few degrees warmer than in the open outside, but from the temperature reactions described above it appears unlikely that this difference has any influence on the behaviour of the mosquito seeking a blood meal or looking for a resting place. This contention is also supported by our previous observations that at night there is no regular difference in temperature between the air inside a typical cow house and that of a human habitation, temperature alone, therefore, being of little use in guiding the mosquito to man or his habitation.



Although the reactions to temperature fail to explain or simplify any of the mosquito's nocturnal activities, they might possibly have an influence on the behaviour during the day. Repeated observation has shown that blood fed and gravid females disturbed in their daytime resting places by day show no tendency to leave the house, and such behaviour might be influenced or controlled by a strong avoidance of the higher temperature outside the house, a vital reaction which would prevent the mosquito from entering an atmosphere with a dangerously high temperature.

#### THERMAL DEATH POINT OF ADULTS.

In the previous section, it was shown that the most outstanding reaction of adult *A. minimus* to choice of temperature was the marked avoidance of high temperatures. It will now be interesting to see exactly what temperatures proved rapidly fatal to the mosquitoes, and to find if this thermal death point is influenced by different relative humidities.

The simple apparatus used to study this consisted of a circular glass dish about 7 inches in diameter and about 3 inches deep. A square plate of glass with a small central hole  $\frac{1}{2}$  inch in diameter formed the roof, the junctions being sealed with a thin film of vaseline. Inside the glass chamber were a few shallow dishes containing different salt solutions according to the relative humidity required, and on these rested a false floor of perforated zinc covered with muslin. The whole apparatus was partly immersed in a water bath. About 10 to 20 blood fed *A. minimus* females were introduced into the chamber through the small hole in the roof, and the water bath was heated so that the temperature inside the closed apparatus rose slowly, at the rate of about 5°C. per hour. A circular piece of filter paper rested on the false floor and was attached to a thread which passed through the small hole in the glass roof; by raising and lowering the paper at intervals the air inside was mixed and kept at a uniform temperature.

There were slight differences between different experiments, but in general the behaviour was as follows:—As the temperature approached 35°C. and 36°C., there was great activity, with most females in flight. Between 36°C. and 37°C. most of the mosquitoes settled, although a few still remained active; 37°C. to 38°C., was the fatal zone within which the majority of females died after an exposure of 5 or 10 minutes, very few surviving short exposures at 38°C. The experiments were carried at different relative humidities from 25 per cent to 90 per cent, but the general onset of death and the thermal death point was not affected by relative humidity within that range. The thermal death point of recently emerged and unfed females was much the same as that of fully blood fed mosquitoes.

In the same apparatus recently blood fed *A. vagus* had a thermal death point between 40°C. and 41°C., showing a much greater resistance to high temperatures than *A. minimus*.

It is interesting to note that a corresponding difference in susceptibility to high temperatures is also found in the larvæ of these two species, larvæ of *A. minimus* having a thermal death point of 41°C., compared with that of *A. vagus* which is nearer 45°C.

#### LABORATORY EXPERIMENTS ON LONGEVITY OF *A. MINIMUS*.

Although it is so important to have some idea of the longevity of anophelines, particularly of the vector species, it is extremely difficult to get



accurate information on this subject. The mortality of mosquitoes kept in captivity may give misleading ideas about conditions in the field, although, on the other hand, controlled laboratory experiments with different species, or at different seasons, should give results of comparative value.

So far the only promising method of finding the maximum and average length of life under natural conditions appears to be that of Afridi *et al.* (*loc. cit.*), involving large scale experiments with dusted mosquitoes. For various reasons this method could not be applied to *A. minimus*, and we were forced to fall back on laboratory observations.

As many pupæ and full grown larvæ as possible were collected from breeding places, and the largest numbers of males and females which emerged in two successive days were put in a mosquito-netting cage measuring  $2 \times 2 \times 2$  feet in the insectary. The adults were supplied with dark resting places and raisins or sugar solution. Every second or third night they were given the opportunity of feeding on the author's arm, and they usually fed freely. Dead mosquitoes were removed every day, and a continuous record was kept of temperature and humidity inside the insectary. In the following description, the data are condensed to show the number of mosquitoes alive at the end of each week. At the end of the experiments, it was found that a few mosquitoes were not accounted for, probably due to small jumping spiders pulling the dead bodies through the netting, and an allowance was made for the slight error thus introduced.

The first two experiments were carried out after the end of the rains, at about the peak period of abundance and infectivity rate of *A. minimus*.

The third experiment was carried out during a hot dry period at the end of the cold weather, at a time when the output of adults from cold weather breeding places is at a maximum.

*Experiment 1.* Forty females and 26 males which emerged from pupæ on 21 and 22.x were put in a cage in the insectary, as described above. The numbers alive at the end of each week were as follows:—

		1st week.	2nd week.	3rd week.	4th week.	5th week.
40 females	..	32	24	16	12	3
26 males	..	19	17	4	2	0

After the end of the fifth week one female died on the 36th day and 2 on the 37th day. The last male died on the 33rd day.

*Experiment 2.* Forty females and 25 males which emerged on 23.x and 24.x were put in a large cage and offered a blood meal about every fifth day. Five females survived to the end of the fifth week, but only one was left at the end of the sixth week; this survived for another week, dying on the 49th day. The last male died on the 38th day. Combining these two experiments we see that out of 80 females, 8 survived for five weeks.

The temperature and humidity conditions during the seven weeks of these two experiments are shown in Table VII.

*Experiment 3.* Fifty-five females and 37 males which emerged from pupæ on 13 and 14.iv were put in a large cage as above, and given the opportunity



TABLE VII.

		1st week.	2nd week.	3rd week.	4th week.	5th week.	6th week.	7th week.
Mean ..	Maximum temperature, °C. . .	25.6	24.6	22.3	22.0	20.7	20.7	20.5
	Minimum temperature, °C. . .	20.0	16.5	17.3	17.0	13.4	13.0	12.6
Mean ..	Maximum relative humidity, per cent.	92	98	99	99	100	99	100
	Minimum relative humidity, per cent.	67	69	82	80	75	77	77

of a blood feed every third or fourth day. The numbers alive at the end of each week were as follows :—

		1st week.	2nd week.	3rd week.	4th week.
55 females	..	19	6	4	0
37 males	..	8	1	0	0

Of the 4 females which were alive at the end of the third week, 2 died on the 22nd day and 2 on the 24th day. The last male died on the 17th day. The climatic conditions in the insectary during this experiment are shown in Table VIII.

TABLE VIII.

		1st week.	2nd week.	3rd week.
Mean ..	Maximum temperature, °C. . .	27.4	27.5	26.6
	Minimum temperature, °C. . .	23.3	22.5	23.1
Mean ..	Maximum relative humidity, per cent.	85	81	86
	Minimum relative humidity, per cent.	73	68	7

The most striking feature of the dry weather experiment was the very heavy mortality of both males and females in the first few days after emergence, and the results suggest that the mosquito's expectation of life is much higher in the humid period after the rains than in the hot dry spells which frequently occur between the end of the cold weather and the beginning of the rainy season. This suggestion is supported by the observation that in these hot dry spells batches of adults sent by post show a higher mortality than at any other time of the year.

But whether or not these laboratory experiments indicate the true state of affairs is a different matter, and it would need much more convincing proof before one could use this as a basis for explaining the seasonal infectivity of *A. minimus* or other epidemiological phenomena.



## SUMMARY.

(1) The main daytime resting places of *A. minimus* in Assam and North Bengal are inside dark native houses and coolie huts. The temperature, humidity, and light conditions of the resting places are described and compared with those of (a) a standard Stevenson screen, and (b) a typical cow house.

(2) During the hot damp monsoon season, *A. minimus* takes two days to digest its blood meal and develop its ovaries, ovipositing on the second night after feeding. In cold weather conditions, this period is increased to from four to six days.

(3) At sundown there is a strong attraction to light, and most mosquitoes leave the house at this time. There is no similar reaction at dawn.

(4) Marking and other experiments show that there is a considerable daily turnover of the population of *A. minimus* in houses.

(5) About 90 per cent of blood feeding takes place after midnight under natural conditions, and there is very little feeding activity during the two or three hours after sundown. After ovipositing, *A. minimus* returns for another blood feed on the same night.

(6) *A. minimus* lays more eggs per batch in the monsoon than in the cold weather.

(7) *A. minimus* refused to mate in captivity, and in unfertilized females there is no development of ovaries after a blood meal. In nature, i.e., in fertilized females, one blood meal is sufficient for egg laying.

(8) Blood feeding takes place throughout the year, and there is no state of hibernation in the cold weather.

(9) In captivity, *A. minimus* feeds readily on various animals, but cow appears to be a more suitable alternative host than goat.

(10) *A. minimus* shows no marked reaction when exposed to a choice of different degrees of atmospheric humidity. When exposed to a choice of temperature, the most marked reaction is a strong avoidance of higher temperatures; this reaction is well marked above 25°C. in hungry females, and above 30°C. in blood fed ones.

(11) The fatal zone of temperature is 37°C. to 38°C. for *A. minimus* adults at relative humidities from 25 to 90 per cent. *A. vagus* is more resistant, with a fatal zone at 40°C. to 41°C.

(12) Laboratory experiments suggest that in *A. minimus* the expectation of life is greater at the end of the rainy season than it is in the hot dry spells before the rains.

## REFERENCES.

- AFRIDI, M. K., MAJID, ABDUL, and J. Mal. Inst. Ind., 3, 1, p. 23.  
 SHAH, IMDAD ALI (1940).  
 CHANG, T. L. (1940) .. .. Chin. Med. Jour., 55, 2, p. 218.  
 MAJID, S. A. (1937) .. .. Rec. Mal. Surv. Ind., 7, p. 105.  
 DE MEILLON, B. (1937) .. South Afric. Inst. Med. Res. Publication. No. XL, 7, p. 313.  
 RAMSAY, G. C., CHANDRA, S. N., and Rec. Mal. Surv. Ind., 6, 1, p. 49.  
 LAMPRELL, B. A. (1936).  
 RICE, E. M., and MOHAN, B. N. Ibid., 6, p. 557.



- ROY, D. N. (1940) .. *J. Mal. Inst. Ind.*, **3**, 4, p. 449.  
 RUSSELL, P. F. (1931) .. *Phil. Jour. Sci.*, **36**, p. 639.  
     *Idem* (1932) .. *Proc. Ent. Soc. Washington*. **34**, No. 8, p. 131.  
 THOMSON, R. C. M (1938) .. *Bull. Ent. Res.*, **29**, 2, p. 125.  
     *Idem* (1940) .. *J. Mal. Inst. Ind.*, **3**, 2 and 3, p. 265.  
 TOUMANOFF, C. (1936) .. *L'Anophélisme dans l'Extrême-Orient*. Paris.  
 VISWANATHAN, D. K. (1941) .. *J. Mal. Inst. Ind.*, **4**, 1, p. 35.







# STUDIES ON THE BEHAVIOUR OF *ANOPHELES MINIMUS*.

## Part VI.

### OBSERVATIONS ON THE COLD WEATHER BREEDING PLACES.

BY

R. C. MUIRHEAD THOMSON.

(*Research Worker supported by the Royal Society and the London School of Hygiene and Tropical Medicine.*)

[September 10, 1941.]

### CONTENTS.

	PAGE.
A. INTRODUCTION .. .. .	247
B. GENERAL .. .. .	248
C. THE TEMPERATURE OF COLD WEATHER BREEDING PLACES ..	248
D. THE OUTPUT OF ADULTS FROM PERENNIAL RIVERS IN THE COLD WEATHER	249
E. COLD WEATHER BREEDING PLACES IN THE DOOARS ..	252
F. DISCUSSION AND PRACTICAL CONSIDERATIONS .. ..	253
SUMMARY .. .. .	254
REFERENCES .. .. .	255

### A. INTRODUCTION.

DURING the last ten or fifteen years there have been rapid advances in the study and control of malaria in Assam and North Bengal, and the general habits and breeding places of *A. minimus*, the most important vector species, are now familiar not only to most medical officers but also to the large body of malaria surveyors throughout that area. The building up and co-ordination of this antimalaria work, particularly in tea districts, are principally due to the efforts of Dr. G. C. Ramsay, Deputy Director, Ross Institute of Tropical Hygiene.

In order to reap the full benefit from such an intensive and extensive anti-malaria scheme, it is desirable that there should be agreement on the general methods to be applied, and that there should be no opposing schools of thought or action. Such a state of affairs is usually traceable to incomplete knowledge



of the bionomics of the vector species. An example of this is the question of cold weather breeding of *A. minimus* and the existence of divided opinions about the exact rôle of the breeding places at that time of the year.

Earlier workers, Manson and Ramsay (1932; 1933), suggested that larval development in the cold weather was so slowed down that a state of hibernation occurred. Rice and Mohan (1936), however, showed convincingly that *A. minimus* breeding continues actively, although more slowly, throughout the cold weather months, and that the larvæ found in perennial streams do not remain in hibernation but emerge as adults. In this respect, it is important to distinguish between these research findings, which are well established and have been confirmed in essential facts by the present author, and the recommendations based on these findings (Rice, 1935) which are not proven and are open to criticism.

In the present paper an attempt is made, in the light of further observations, to put the cold weather breeding places in their proper perspective, and to compare the results obtained with the existing methods of control. Much of this consists of going over old ground and repeating familiar facts, but this is felt to be a necessary procedure in a subject still open to controversy.

Most of the detailed work was carried out in the Assam Valley, but observations were also made in the Eastern Doonars, where conditions are modified by the different type of terrain.

## B. GENERAL.

After the end of the rainy season in Assam, numerous grassy-edged streams and seepages, which have been ideal monsoon breeding places, begin to dry up, and eventually *A. minimus* has to look elsewhere for a breeding ground. At the same time, in the large rivers and perennial streams, the level of the water is falling, there are no longer frequent spates, and with the fall in current velocity and the appearance of vegetation at the edges conditions become suitable for regular breeding. Although there are numerous intermediate streams in which small numbers of larvæ are found both in the monsoon and during part of the cold weather, this does not alter the fact that, in general, the two most important types of breeding places, which we have to consider in Assam, are the small monsoon streams and seepages, typified by the open, grassy-edged tea garden drains, and the large perennial rivers in the cold weather. In the present paper, we are principally concerned with these perennial rivers, but due attention will also be paid to those areas in which the principal cold weather breeding place is of rather a different nature.

## C. THE TEMPERATURE OF COLD WEATHER BREEDING PLACES.

In a previous communication (Thomson, 1940), it was shown, by means of laboratory experiments on the effect of temperature on eggs and pupæ, that throughout the greater part of the cold weather the water temperatures are such that rapid development of these stages at least takes place, and that successful development still takes place at 16°C., the lowest mean temperature likely to be encountered in a breeding place in the coldest part of the year.

Measurements made in Upper Assam during the first two weeks of January, usually the coldest time of the year, showed that a minimum water temperature below 15°C. only occurred on the coldest night in the year (with a minimum



screen temperature of 43°F. or 6.1°C.), and that by the second week of January the minimum water temperature seldom fell below 16°C. From that time onwards there is a steady rise in water temperature, and its effect on development can best be appreciated from the course of the temperature-development curve previously described (Thomson, *loc. cit.*, Graph 3, p. 344).

From these observations it appears that there is no time of the cold weather when the water temperature is low enough to prevent hatching of eggs and emergence of adults from pupæ. It seems extremely likely that these results also apply to the development of larvæ, but as it was not possible to carry out similar exact experiments on the effect of temperature on larval development, this assumption cannot be taken for granted without further evidence. As these experiments do not preclude the possibility that low temperatures, while not preventing development of pupæ, may possibly prevent larvæ from pupating, it is necessary to show that pupation actually takes place in the breeding place. That pupation really does take place is amply proved by the fact that pupæ are regularly found in the breeding place throughout the entire cold weather, and on temperature considerations alone, therefore, the continual production of adults from the breeding place throughout this period is indicated. Using entirely different methods, therefore, the findings and conclusions described in this section entirely corroborate those of Rice and Mohan (*loc. cit.*). It now remains to extend these fundamental observations and try to estimate the importance of cold weather breeding places by gauging the density of breeding and the output of adults.

From the temperature figures described above we would expect, with the rise of water temperature from January onwards, a rapid increase in rate of development of all stages, and it will, therefore, be essential to have a clearer idea of the importance of the perennial river at different periods throughout the cold weather.

#### D. THE OUTPUT OF ADULTS FROM PERENNIAL RIVERS IN THE COLD WEATHER.

The most convincing way of estimating the relative importance of a particular breeding place is to find its output of adult mosquitoes. A method which has been applied with success by other workers is to put mosquito nets over standard areas of the breeding place, all the adults which emerge being trapped inside the net where they are collected each day (Russell *et al.*, 1940). Where breeding is mainly confined to the grassy edge of running water—as with *A. minimus*—this ideal method is not practicable: first, because larvæ are usually so sparsely distributed that an inconveniently large net or number of nets would be required. Secondly, any mosquito in the output net in contact with the bank of the river would be exposed to the attacks of ants, jumping spiders, etc., in a way that might seriously affect the results. Thirdly, in the type of breeding place with which we are principally concerned, the risk of interference with the net or the chance of its being stolen is considerable.

In view of these obvious drawbacks, a new method had to be devised to suit the present circumstances. This was to collect all pupæ in a measured length of grassy edge, transfer them to water in the laboratory with approximately the same mean temperature as the river, and record the number of female *A. minimus* produced and the number of days which elapsed before



emergence. In practice, two variations of this method were employed: in the first, a length of continuous grassy edge, extending from 30 to 200 feet depending on the density of breeding, was thoroughly searched twice by two collectors using white enamelled frying pans, all larvæ and pupæ being collected. The larvæ which pupated on the day of capture were included with the other pupæ. When the experiment was repeated, a completely new length of edge was chosen. In the second method, pupæ alone were collected from the measured length of bank, all larvæ being replaced. Employing the latter method, the same length of edge could be used several times, to give an output estimate. As large numbers of larvæ were required for other experiments, the first method was the one usually adopted.

The disadvantages of this method are: (i) One cannot be certain that all the pupæ in a measured length are actually captured by the collectors. This sampling error is obviated as far as possible by going over the same area twice with two expert collectors. (ii) The method does not take into account pupal mortality in the field. We do know, however, that pupal mortality in the laboratory is almost nil, and once a larva has pupated it is exceedingly rare for successful emergence not to follow in due course. The advantages of the method are: (i) that even when the output is very low it is possible to arrive at a rough estimate by sampling longer and longer lengths of grassy edge; and (ii) one can estimate the output from breeding places remote from the laboratory or field station, which it would be inconvenient to visit every day. In recording these results, only female *A. minimus* were taken into account, and the results are expressed as 'the number of feet of continuous grassy edge which produce one female per day'. For example, from pupæ of different species collected in 50 feet of edge, 8 female *A. minimus* emerged within the next 3 days; the output therefore, was one female per day from 19 feet of grassy edge. It must be emphasized that the figures only refer to the length of grassy edge, and not to the length of river bank.

These methods were applied to two large rivers in the Sibsagar Division of Upper Assam. The first river, the Desoi or Bhogdoi, emerges into the plains through dense forest at the foot of the Naga Hills, and flows in a meandering course for 15 or 20 miles across flat country towards the Brahmaputra (Plate XXVI, fig. 1). The first part of its course after its emergence from the foot-hills is through an area of well-controlled tea gardens. During and after the rainy season, it is difficult to find continuous dense breeding of *A. minimus* anywhere in this area, owing to the vigorous antimalaria operations then in progress. During the cold weather, the monsoon breeding places dry up and the Desoi becomes increasingly important as a breeding place. The fall in level of the river leaves long stretches of river edge bare and exposed and therefore harmless, but in other parts of the river there are stretches of thick grassy edge in which larvæ eventually reach a density seldom encountered elsewhere in the area. At intervals from January to April 1939 larvæ were collected from different sections of the river and estimates of the output of *A. minimus* were made; the results are shown in the table. Although conditions vary considerably from year to year, the density of larvæ and pupæ in April 1939 was not abnormally high, as very similar conditions were encountered again in the same river in April 1941.

The second river, the Dunsiri, is a much larger perennial river which follows a somewhat similar but longer course from the foot-hills to the



Brahmaputra (Plate XXVII, fig. 2). Collections were made where this river flows through an extensive stretch of dense forest—the Nambar Forest—sparsely populated and quite uncontrolled. At the end of the rainy season, very few *A. minimus* larvæ were found in this area, probably due to the scarcity of suitable breeding places not covered with heavy jungle; but during the cold weather breeding was found to occur along the thick 'grassy edge' of this broad jungle river, the grassy edge in this case consisting entirely of *Polygonum* sp. Estimates of the output were made in February and March 1939 and again in January 1940. The results are shown in the table.

In order to appreciate the full significance of these figures, it must be borne in mind that *A. minimus* is a highly efficient vector, and even a small population

TABLE.

*The output of A. minimus females from perennial rivers, January to April.*

Date.	Length of 'grassy' edge (feet).	Number of <i>A. minimus</i> larvæ.	Number of pupæ (♀ <i>A. minimus</i> ).	Length of grassy edge producing one female <i>A. minimus</i> per day (feet).
I. Desol river.				
9.ii.39	200	76	3	266
11.ii.39	120	127	3	160
16.ii.39	200	..	4	150
18.ii.39	200	..	8	75
21.ii.39	70	57	1	120
27.iii.39	63	103	7	18
3.iv.39	100	408	30	7
6.iv.39	60	488	16	5
11.iv.39	103	467	32	8
II. Dunsiri river.				
23.i.40	90	314	18	25
23.ii.39	50	182	8	19
2.iii.39	41	244	6	21
	44	120	14	10
	31	77	5	19

can maintain a high degree of malaria. The density of breeding seldom approaches, and cannot be compared with, that recorded for species such as *A. culicifacies* elsewhere in India.



As both 1939 and 1940 were years of unusually low rainfall, it was not possible to make corresponding estimates for monsoon breeding places, since the distribution of larvæ was very scattered and irregular. From purely empirical observations made during 1938, a year which was favourable for dense breeding in the rains, it would appear that such a high output of female *A. minimus* per unit length of grassy edge is unlikely to occur in monsoon breeding places until the rains have ceased. But this is amply compensated for by the vastly greater number of breeding places available at that time of the year.

A third large river, which follows a similar course from the Naga Hills to the Brahmaputra, was investigated. This river, as described before (Thomson, *loc. cit.*), had completely bare, exposed edges throughout most of its length; in the total absence of larvæ, much less of pupæ, no estimate of its apparently insignificant output could be made (Plate XXVIII, fig. 3).

A good idea of the rapid increase in density of larvæ and output of adults can be gained by comparing the first two catches in the Desoi river, those of February 9 and 11, with the last two collections made two months later, on April 6 and 11. At the beginning of February, the mean density of larvæ was 63 per 100 feet, and the mean output of adult females 1 per day from 213 feet of grassy edge. By the beginning of April, the mean density of larvæ was 585 per 100 feet of edge, and the mean output of females 1 per day from 6½ feet of edge. During this two-month period, therefore, when breeding is frequently allowed to carry on unchecked, there is roughly a ten-fold increase in larval density and a thirty-fold increase in the output of female *A. minimus*.

For the Dunsiri river, figures are not complete enough to give a similar comparison, but the 1940 estimates show a high output as early as January, which is all the more striking in view of the thinly populated forest through which the river flows at this point.

In some of these districts, where larval control is vigorous and conscientious for most of the year, the treatment of perennial rivers is not taken up till the middle of March or later, by which time the river is producing large numbers of *A. minimus* adults. Not only are these adults potential producers of later generations which can quickly make use of monsoon breeding places, but it is also possible for this generation to be directly responsible for new infections in April or even earlier. It is clear that such a breeding place cannot be overlooked in any antimalaria scheme.

#### \* E. COLD WEATHER BREEDING PLACES IN THE DOOARS.

As we go westwards from the Assam Valley to the Dooars of North Bengal and the Darjeeling Terai, we find rather different conditions in the cold weather. Owing to the stony nature of the soil, the perennial rivers have an entirely different appearance from those described above. Many large rivers dry up completely in the cold weather; those which run throughout the year have the water mostly confined to a stony, gravelly, or sandy bed. Along a bare edge of this nature, with complete absence of vegetation, *A. minimus* is seldom found. It appears, therefore, that these large perennial rivers in the Dooars can play only a minor part as cold weather breeding places, as there are comparatively few situations in which the water remains in contact with a grassy edge. In parts of the Eastern Dooars, lying in the area close to the foot-hills, there are



PLATE XXVI.



Fig. 1. The Desoi (Bhogdoi) River in Upper Assam where estimates of the adult output were made in the cold weather.



PLATE XXVII.

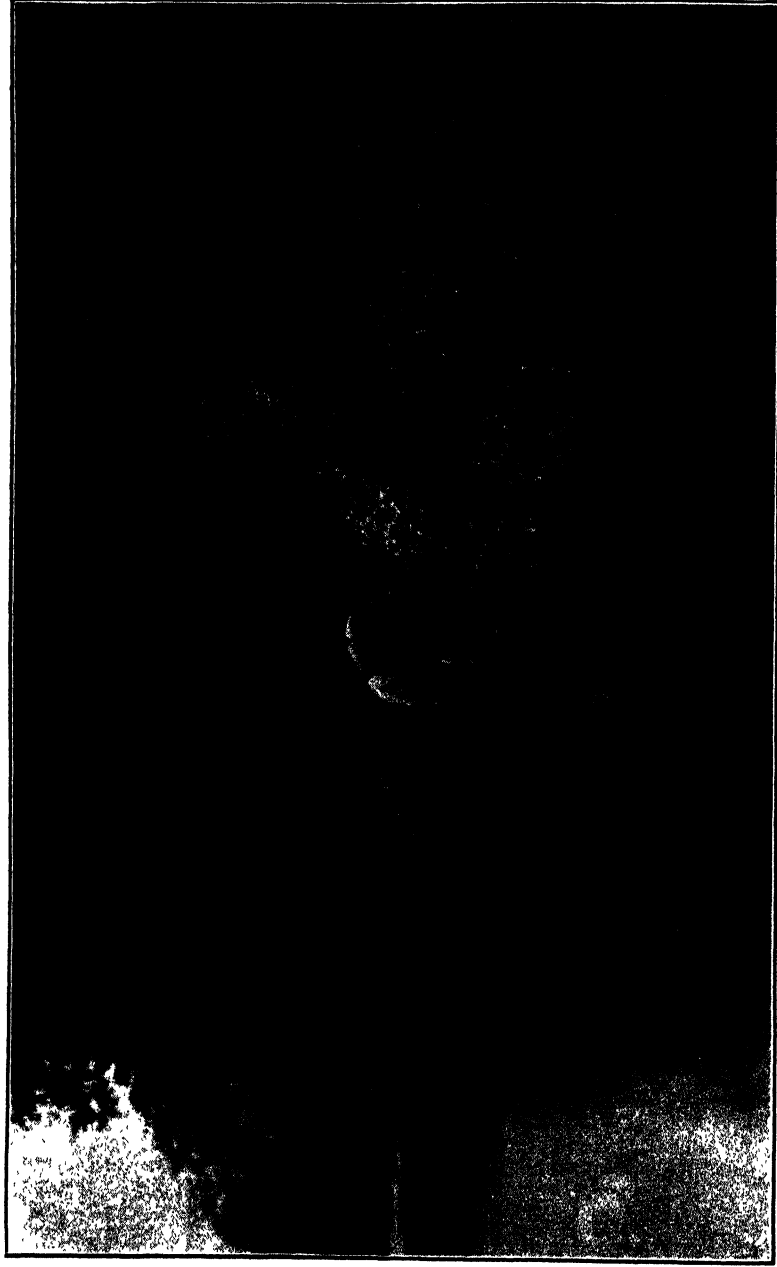


Fig. 2. The Dunsiri River flowing through dense forest where estimates of adult output were made.



PLATE XXVIII.

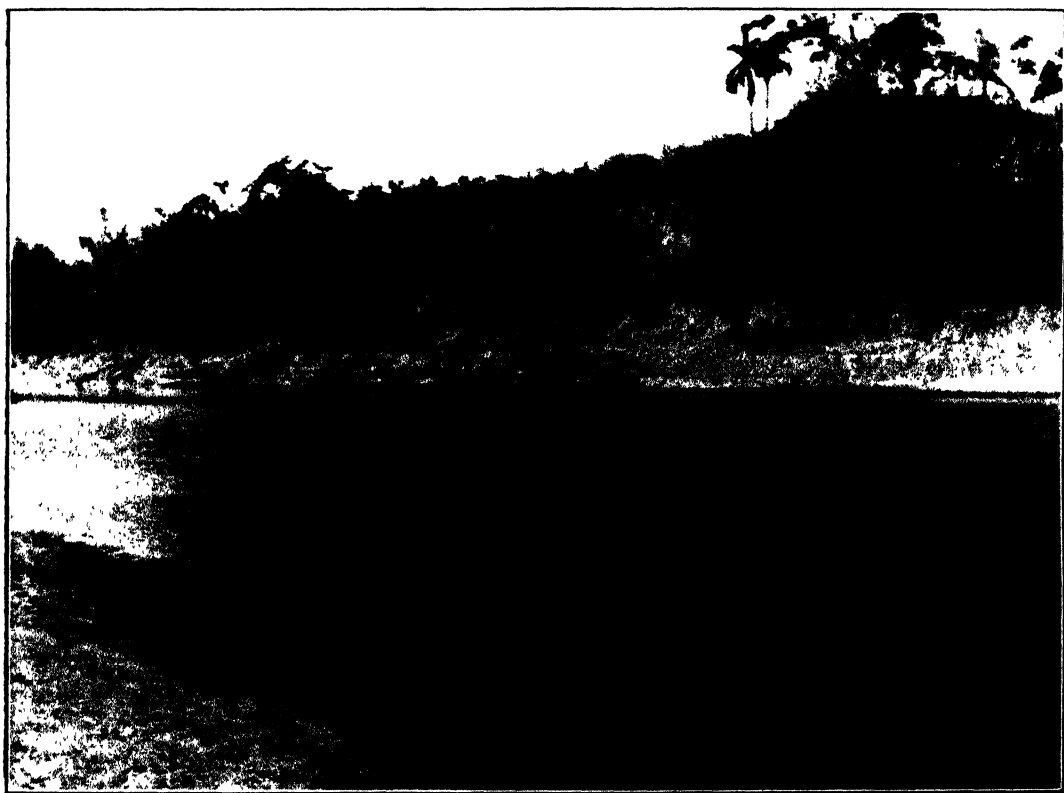


Fig. 3. A perennial river with bare edges where the output of adults was insignificant and too low to estimate.







areas where in the dry season there is no surface water for many miles, and the nearest perennial rivers, with stony beds, are quite unsuitable as breeding places. And yet many of these dry gardens are very malarious (Harrison and Ramsay, 1933; Ramsay, personal communication). At first sight this is very puzzling, and the apparent absence or extremely low density of breeding seems to contradict the findings in Assam and to relegate cold weather breeding to a position of negligible importance. However, only a few miles south of this dry zone there is an area of perennial seepages with clear running water and grassy edges, and also broader grassy-edged streams formed from these seepages. From the number of *A. minimus* larvæ and pupæ found in these seepages, it seems that they are of first importance as cold weather, or dry weather, breeding places. Although no estimates of output from these breeding places were made, the part they play is amply demonstrated by the large number of blood-fed adults regularly recorded from the adjacent coolie lines in March; nearly every house yielded positive results and catches of 10 to 15 female *A. minimus* in a single coolie hut were not unusual.

It appears from this and from other evidence that the behaviour of *A. minimus* in the Dooars is essentially the same as in Assam, despite the striking difference in the nature of the country, and that cold weather breeding is just as important a feature there as elsewhere. The complete absence of cold weather breeding places within the driest zones is misleading, because the area of perennial breeding places with its high output of adults at the edge of these dry zones seems to be an ideal focus from which *A. minimus* extends into the dry zone with the advent of the monsoon and the appearance of suitable breeding places.

In Assam, there is frequently a gap between the drying up of the monsoon breeding places and the regular appearance of larvæ in the perennial river. But, in the Dooars, it appears that the same breeding place may continue to produce *A. minimus* throughout the entire year without a break. Such a cold weather breeding place starts the season with a distinct advantage as a source of production of this species, and it would be interesting to know if this results in a higher output of adults in the early part of the year than occurs in Assam.

## F. DISCUSSION AND PRACTICAL CONSIDERATIONS.

From these observations and figures it appears that any scheme of anti-malaria work directed against *A. minimus* should, if possible, include the control of cold weather breeding places from the beginning of the year, and that a common practice of allowing control to lapse during the cold weather till March, or even later, is unscientific and unwise. As the large perennial rivers are the main cold weather resorts of *A. minimus* in Assam, they have received most attention in this investigation, but small perennial streams may be equally important in some localities, particularly in foot-hill regions. In the Dooars area, where the perennial rivers are mostly confined to stony, gravelly, or sandy beds in the dry months, the amount of grassy edge is very limited and such rivers are not of primary importance; in that area grassy-edged perennial seepages and small streams produced by them should claim first attention in the cold weather and the hot dry season.

In the perennial rivers in Assam, it must be emphasized that the amount of river edge with sufficient grass or vegetation to form an ideal breeding place may constitute only a small proportion of the total river bank. Where such a



grassy edge is absent, leaving the bank bare and exposed, *A. minimus* larvæ are scarcely ever found, and one river has been described in which this condition prevails throughout almost its entire course across the plains. These observations have already been confirmed and amplified by laboratory and field experiments, in which it was found that the removal of vegetation and the exposure of the bare edge to sunlight constitutes a simple and extremely effective method of control (Thomson, *loc. cit.*). This seems to be a procedure which might lend itself especially well to perennial rivers, particularly as no other natural methods of controlling these breeding places are widely practised. The well-known and well-established method of control by 'shade' (i.e., planting permanent hedges of shading plants which render the breeding place unsuitable by destroying the vegetation underneath and making the edges of the stream permanently bare), which is so effective for narrow streams and open garden drains, cannot be applied to broad streams or perennial rivers. As the amount of grassy edge is usually not extensive, its removal would not be tedious, and the method would be facilitated by the absence of any great fluctuations in the level of the river throughout most of the cold weather. An objection frequently raised to this suggestion is that removing the vegetation would expose the edges to erosion by the current. As much of the river edge is bare already, and as those parts of the bank which it is particularly important to preserve are already protected by bamboo breakwaters, this objection is largely groundless.

In the case of some smaller streams or *jans* with a tortuous course and an extensive shore line this method might be laborious, but would certainly be worth a trial. In rural areas, where money is scarce and antimalaria operations a luxury, this is probably the only method with any chance of success.

Although the importance of cold weather breeding places has been stressed throughout this paper, it must not be imagined that the control of such places is the be-all and the end-all of operations against *A. minimus*. This discussion has been principally concerned with trying to place cold weather breeding in its proper perspective and to estimate its importance in the general scheme. On these findings it would appear that any scheme of antimalaria work should, if possible, take into account and include the control of cold weather breeding places from the beginning of the year, or at least the beginning of February, until the time when they are regularly flushed out by the rain, by which time attention must naturally be directed towards the control of monsoon breeding places.

#### SUMMARY.

1. The question of cold weather breeding is discussed in the light of recent findings.
2. The development of eggs, larvæ, and pupæ continues to proceed at the lowest mean temperature likely to be encountered in cold weather breeding places.
3. The output of adult *A. minimus* from a perennial river is estimated by a new method based on collections of pupæ.
4. In suitable breeding places, there may be a considerable output of females in January, the coldest month of the year.
5. In one typical perennial river, there was a ten-fold increase in larval density between the early part of February and the early part of April.



During this two-month period, there was a thirty-fold increase in the output of female *A. minimus* from the same breeding place.

6. In the dry zones of the Dooars, the most important cold weather breeding places are perennial seepages and small streams formed from them.

7. In Assam, breeding in many perennial rivers can be simply controlled by removing the grassy edge and exposing the bare edge to sunlight.

8. It is considered that any antimalaria scheme should, if possible, include control of cold weather breeding places from the beginning of the year, or the beginning of February at the latest, until they are regularly flushed out by the first heavy rains, when attention must naturally be turned to monsoon breeding places.

#### REFERENCES.

- HARRISON, C. C., and RAMSAY, G. C. *Jour. Trop. Med. Hyg.*, **36**, p. 33.  
(1933).
- MANSON, D., and RAMSAY, G. C. *Rec. Mal. Surv. Ind.*, **2**, 3, p. 143.  
(1932).
- |                                    |   |                |                       |
|------------------------------------|---|----------------|-----------------------|
| <i>Idem</i>                        | (1933).   | <i>Ibid.</i> , | <b>3</b> , 3, p. 479. |
| RICE, E. M. (1935)                 | .. ..   | <i>Ibid.</i> , | <b>5</b> , 4, p. 371. |
| RICE, E. M., and MOHAN, B. (1936). |   | <i>Ibid.</i> , | <b>6</b> , p. 557.    |
| RUSSELL, P. F., and RAO, H. R.     | <i>J. Mal. Inst. Ind.</i> , <b>3</b> , 4, p. 427. |                |                       |
| (1940).                            |   |                |                       |
| THOMSON, R. C. M. (1940)           | ..  | <i>Ibid.</i> , | <b>3</b> , p. 265.    |







## ON THE MICROSPORIDIA INFESTING SOME ANOPHELINES OF INDIA.

BY

P. SEN, M.Sc. (Cal.), Ph.D. (Lond.), D.I.C.  
(Entomologist, Bengal Public Health Department, Calcutta).

[September 16, 1941.]

### INTRODUCTION.

THE Microsporidia have so far received inadequate attention from Indian zoologists, although some of them have been long known for their devastating effects on human economy. The pebrine disease, which has wrought so much damage to the silk industry in almost every silk-growing country, is caused by one of the Microsporidia, *Nosema bombycis* Nägeli. Bhatia (1938) has recently pointed out the insufficiency of our knowledge of this group of Protozoa, and has remarked that the 'Microsporidia are responsible for causing devastating epidemics in silkworms, honey-bees and certain fishes'. There is also reason to believe that these parasites bring about a heavy mortality among the *Anopheles* population in ponds, and can, therefore, be looked upon as natural enemies of mosquitoes.

So far, the only authors to give an authenticated report on the infestation of Indian anophelines by this group of Protozoa are Kudo (1929) and Iyengar (1929). Kudo, working in America on material submitted by Iyengar, described three species, all belonging to the genus *Thelohania*, of which two were new. In this paper, the author has been able to confirm two of his species, while a third, new to science, is now described for the first time.

### MATERIAL AND METHODS.

The microsporidian parasites here recorded were found infesting the larvæ of the following species of anophelines:—*Anopheles hyrcanus* var. *nigerrimus* Giles, *A. annularis* Van der Wulp, *A. ramsayi* Covell, *A. varuna* Iyengar, *A. subpictus* Grassi and *A. vagus* Dönitz. The entire collection of infested larvæ was made from localities in the vicinity of Calcutta.

The developmental stages of the microsporidian parasites were studied both in freshly prepared unstained smears and in permanent stained preparations.



The permanent preparations were made in the following way:—The infected larvæ were crushed and a smear made on the slide, fixed in Schaudinn's or in alcoholic Bouin's fluid, stained by Delafield's or iron alum hæmatoxylin and mounted in the usual way. Microtome sections of  $6\mu$  thickness were also cut of the parasitized larvæ, stained by Ehrlich's hæmatoxylin and examined under an oil immersion lens.

## RECORD OF OBSERVATIONS.

The preparations studied showed at least three different Microsporidia belonging to the genus *Thelohania*, which were responsible for the diseased condition of the larvæ. A brief description of the different species noted is given below.

### 1. *Thelohania legeri* Hesse.

*Thelohania legeri* is perhaps the commonest Microsporidium occurring in anopheline larvæ, the parasites being recorded from *A. subpictus*, *A. vagus*, *A. hyrcanus* var. *nigerrimus*, *A. ramsayi*, *A. annularis* and *A. varuna*. In a smear of the larval fat-body, the octosporous stage of the parasites, as also the isolated spores, are clearly seen. On puncturing a parasitized larva the spores escape, either separately (Plate XXIX, fig. 1), or in groups of eight (Plate XXIX, figs. 2, 3), and, rarely, in groups of four. The spores of the species when studied fresh are oval in shape being, on an average,  $5\mu$  in length and  $3\mu$  in breadth. The different phases of *Thelohania legeri* noted by the present author closely agree with those described by Kudo (1924a), who has thoroughly worked out its life history. In India, Iyengar (*loc. cit.*), under the description *Thelohania* sp., has given a fairly detailed account of the species.

### 2. *Thelohania indica* Kudo.

*Thelohania indica* was found heavily infesting the fat cells of the larvæ of *A. ramsayi*. The spores (Plate XXIX, fig. 4) measure about  $5\mu$  in length and  $2.5\mu$  in breadth in fixed preparations, and possess a large nucleus at one end. The young schizont (Plate XXIX, fig. 5) has a compact nucleus. The parasite assumes an elongated shape towards the end of its schizogony cycle, and the schizont ultimately produces eight nuclei arranged in pairs (Plate XXIX, fig. 6). This soon divides into binucleated forms (Plate XXIX, fig. 7). The two nuclei of the binucleate schizont then fuse to form a sporont (Plate XXIX, fig. 8). The sporont nucleus subsequently divides into two, four and ultimately eight nuclear stages (Plate XXIX, figs. 9, 10), and an octosporoblast form is reached from which eventually eight spores are liberated. The development of the parasites in the adipose tissue of the larvæ of *A. hyrcanus* has been worked out by Kudo (1929).

### 3. *Thelohania anomala* sp. nov.

The new species of Microsporidium provisionally placed under *Thelohania* was recorded from the adipose tissue of the larvæ of *A. ramsayi*.

In a freshly prepared smear of the fat-body of the larvæ infested with the parasites, the spores measured from  $5.1\mu$  to  $6.1\mu$  in length and  $2.0\mu$  to



2.1  $\mu$  in breadth. If the spore is pressed hard against a slide the breadth becomes 3.0  $\mu$ . The polar end of each spore is tapered (Plate XXIX, fig. 11). The nucleus is situated at the posterior blunt end and close to this there is a vacuole. The young schizont has a compact nucleus with a roundish body (Plate XXIX, fig. 12). The nucleus undergoes a division and a constriction appears in the cytoplasm (Plate XXIX, fig. 13), and two uninucleated daughter schizonts are formed. Some of the schizonts with two nuclei do not divide into daughter schizonts, but their nuclei undergo a further division, the complementary pairs of nuclei being connected with strands, and form a four-nucleated stage (Plate XXIX, fig. 14). The cytoplasm also becomes constricted and ultimately divides into two binucleated parasites (Plate XXIX, fig. 15). The paired nuclei become greatly enlarged and fusion takes place (Plate XXIX, fig. 16) to form a sporont. The uninucleated sporont then divides into two, four, eight, ten or twelve nucleated stages (Plate XXIX, figs. 17, 18), eventually constituting eight to twelve sporoblasts surrounded by a thin membrane (Plate XXIX, fig. 19). Each sporoblast finally gives rise to a mature spore.

Extrusion of polar filament was observed after treating the spores in strong  $\text{HNO}_3$  (conc.). A weaker solution (10 per cent), however, gave negative results. The iodine method was also tried, but was found unsuitable. The polar filament when released by chemical treatment measured 45  $\mu$  in length.

The microsporidian parasite described here closely resembles an unnamed species of *Thelohania* recorded by Missiroli (1929), which also has eight to twelve sporoblasts. The difference lies, however, in the measurement of the spores, which are much larger in the species here described, being 5.1  $\mu$  to 6.1  $\mu$  in length as compared with 3  $\mu$  to 4  $\mu$  in the species recorded by Missiroli. In the structure of the spores the new parasite resembles *T. legeri* Hesse, but it can be readily distinguished from the latter by the somewhat narrower dimension of the spores and the greater number of sporoblasts.

## DISCUSSION.

The present study shows that microsporidian infection among anopheline larvæ is very common. The parasites occur almost throughout the year, but they are particularly prevalent during the period April to September. The thorax becomes opaque in infected larvæ and in advanced cases the abdomen also. The larvæ become very sluggish when heavily infected, and usually die within two to six days in captivity, rarely reaching the pupal stage. This observation is in conformity with that of Kudo (1925). Kudo (1921) was much impressed by the effect of this parasitization, and he saw in these parasites a possibility of effecting a natural control of the mosquito larvæ by inducing the disease among them artificially through the distribution of the infected larval tissue in their breeding places.

As already mentioned, Kudo (1929) recorded three species of *Thelohania*, *T. indica* Kudo, *T. legeri* Hesse and *T. obscura* Kudo from Indian anophelines. The present studies from almost identical material show that *T. indica* and *T. legeri* are widely prevalent, while the existence of an entirely different species, *T. anomala* sp. nov., is now recorded. On the other hand, *T. obscura* was not observed by the present author. The distribution of the various



## 260 *On the Microsporidia infesting Some Anophelines of India.*

species of *Thelohania* among the larvæ of the Indian anophelines, as it stands at the present time, is as given below:—

Parasite.	Host larvæ.
<i>T. legeri</i> Hesse	.. <i>A. barbirostris</i> Van der Wulp. <i>A. hyrcanus</i> var. <i>nigerrimus</i> Giles. <i>A. annularis</i> Van der Wulp. <i>A. ramsayi</i> Covell. <i>A. varuna</i> Iyengar. <i>A. subpictus</i> Grassi. <i>A. vagus</i> Dönitz.
<i>T. indica</i> Kudo	.. <i>A. hyrcanus</i> var. <i>nigerrimus</i> Giles. <i>A. ramsayi</i> Covell.
<i>T. obscura</i> Kudo	.. <i>A. varuna</i> Iyengar.
<i>T. anomala</i> sp. nov.	.. <i>A. ramsayi</i> Covell.

From the above list, it will be seen that at least seven species of *Anopheles* are parasitized by *Thelohania*. Of these, *A. barbirostris*, *A. hyrcanus*, *A. annularis*, *A. ramsayi*, *A. varuna* and *A. subpictus* were long ago incriminated by both Iyengar (*loc. cit.*) and Kudo (1929). *A. vagus* Dönitz, in which infestation by *T. legeri* has been noted here, is thus a new addition to the previously known list of anopheline hosts. The detection of *T. indica* in the larvæ of *A. ramsayi* is also a new record, the species being previously recorded from *A. hyrcanus* only by Kudo (1929).

Besides the species of *Thelohania* from India just discussed, Kudo (1929) records *T. obesa* Kudo from the American mosquito, *A. quadrimaculatus*, and *T. legeri* which is ubiquitous in distribution from *A. bifurcatus*, *A. crucians*, *A. punctipennis*, as also *A. quadrimaculatus*, while about half a dozen more species (*T. opacita* Kudo, *T. obesa* Kudo, *T. pyriformis* Kudo, *T. rotunda* Kudo, *T. minuta* Kudo and *T. grassi* Missiroli) have been described by Missiroli (*loc. cit.*) from the European mosquito, *A. maculipennis*, alone. Some of these species are parasites of a number of culicine species as well.

Missiroli (*loc. cit.*), in addition to the species already mentioned, records a new *Thelohania* with eight to twelve sporoblasts from *A. maculipennis*, resembling *T. anomala* sp. nov. It is not known why Missiroli did not give a name to this new parasite; possibly he was not certain about its systematic position.

Some amount of confusion is bound to arise in placing Microsporidia with eight to twelve sporoblasts such as *Thelohania* sp. of Missiroli and *T. anomala* sp. nov. under the genus *Thelohania*, when one considers the fact that a typical *Thelohania* should have eight sporoblasts only from which eventually eight spores are liberated (Kudo, 1924a; Bhatia, *loc. cit.*). Instances, however, are not altogether rare where a parasite, in spite of possessing a variable number of eight to more spores in a mature sporont, has been designated under *Thelohania*. Thus, in *Thelohania giraudi* Leger some of the sporonts have been known to contain 16, 32 or more spores in addition to the normal octosporous sporonts. Similarly in *T. mulleri* (Pfeiffer), the number of spores may be as great as 16, 24 or 32, while an extreme case is represented by *T. multisporea* (Strickland) parasitic on *Simulium* larvæ, with sometimes as many as 30 to 60 nuclei in a pansporoblast (Kudo, 1924a).







## EXPLANATION OF PLATE XXIX.

(All camera lucida drawings. Magnification  $\times 1350$ .)

- Fig. 1. Spore of *Thelohania legeri* Hesse.
- „ 2. A group of eight spores of *T. legeri* Hesse.
- „ 3. A group of eight spores of *T. legeri* Hesse (a different arrangement).
- „ 4. Spores of *Thelohania indica* Kudo.
- „ 5. Young schizont of *T. indica* Kudo.
- „ 6. Schizont of *T. indica* Kudo with eight nuclei.
- „ 7. Binucleated schizont of *T. indica* Kudo.
- „ 8. Fusion of the two nuclei in the formation of a sporont in *T. indica* Kudo.
- „ 9. Four-nucleated sporont of *T. indica* Kudo.
- „ 10. Fully developed sporont of *T. indica* Kudo.
- „ 11. Spores of *Thelohania anomala* sp. nov.
- „ 12. Young schizont of *T. anomala* sp. nov.
- „ 13. Divisional stage of a schizont of *T. anomala* sp. nov.
- „ 14. Four-nucleated stage of a schizont of *T. anomala* sp. nov. prior to division into two binucleated forms.
- „ 15. A binucleated form of *T. anomala* sp. nov. after the division envisaged in the above stage is completed.
- „ 16. Fusion of the two nuclei in the formation of a sporont in *T. anomala* sp. nov.
- „ 17. Four-nucleated sporont of *T. anomala* sp. nov.
- „ 18. Twelve-nucleated sporont of *T. anomala* sp. nov.
- „ 19. A twelve-sporoblast stage of *T. anomala* sp. nov.



PLATE XXIX.



1.



2.



3.



4.



5.



6.



7.



8.



9.



10.



11.



12.



13.



14.



15.



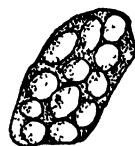
16.



17.



18.



19.







In tracing the life history of the parasites with a twelve-sporoblast stage as in *T. anomala* sp. nov., it is very difficult to say by what actual processes and through how many divisions a stage with this greater number was arrived at from a uninuclear sporont. For the usual octosporous stage, the general belief is that it is evolved through three successive divisions of the primary sporont.

### SUMMARY.

1. Microsporidian infection has been recorded in the larvæ of six species of anophelines, *Anopheles subpictus* Grassi, *A. vagus* Dönitz, *A. ramsayi* Covell, *A. annularis* Van der Wulp, *A. hyrcanus* var. *nigerrimus* Giles and *A. varuna* Iyengar.

2. Three species of Microsporidia, *Thelohania legeri* Hesse, *Thelohania indica* Kudo and *Thelohania anomala* sp. nov., were concerned in the infection. The last-named species is described for the first time.

3. The observations by Kudo (1929) on *Thelohania legeri* Hesse and *Thelohania indica* Kudo from Indian mosquitoes are confirmed.

4. *Thelohania anomala* sp. nov. shows eight to twelve sporoblasts, and a discussion on the various species of *Thelohania* with more than eight sporoblasts is included.

### ACKNOWLEDGMENTS.

The author is greatly indebted to Mr. M. Chakravarty, Lecturer in Protozoology of the University of Calcutta, but for whose assistance the investigations here recorded could not have been completed. He also thanks Dr. H. N. Ray, Systematic Protozoologist at the Imperial Veterinary Research Institute, Muktesar, for many technical suggestions received from him.

### REFERENCES.

- |                          |    |    |  |
|--------------------------|----|----|--|
| BHATIA, B. L. (1938)     | .. | .. | Protozoa: Sporozoa. Fauna of British India, p. 17 and pp. 346-359.   |
| IYENGAR, M. O. T. (1929) | .. | .. | Microsporidian parasites of <i>Anopheles</i> larvæ. <i>Trans. 7th Cong. Far East. Assoc. Trop. Med.</i> , 1927, <b>3</b> , pp. 136-142.  |
| KUDO, R. (1921)          | .. | .. | Studies on Microsporidia with special reference to those parasitic in mosquitoes. <i>J. Morph.</i> , <b>35</b> , pp. 153-193.  |
| <i>Idem</i> (1924a)      | .. | .. | A biologic and taxonomic study of the Microsporidia. <i>Illinois Biol. Monogr.</i> , <b>9</b> , 2 and 3. pp. 61, 67 and 130-163.   |
| <i>Idem</i> (1924b)      | .. | .. | Studies on Microsporidia parasitic in mosquitoes. III. On <i>Thelohania legeri</i> Hesse ( <i>Th. illinoisensis</i> Kudo). <i>Arch. Protistenk.</i> , <b>49</b> , pp. 147-162. |
| <i>Idem</i> (1925)       | .. | .. | Studies on Microsporidia parasitic in mosquitoes. VI. On <i>Thelohania opacita</i> Kudo. <i>J. Parasit.</i> , <b>11</b> , pp. 84-89.   |
| <i>Idem</i> (1929)       | .. | .. | Studies on Microsporidia parasitic in mosquitoes. VII. Notes on Microsporidia of some Indian mosquitoes. <i>Arch. Protistenk.</i> , <b>67</b> , pp. 1-10.                      |
| MISSIROLI, A. (1929)     | .. | .. | Sui microsporidi parassiti del l' <i>Anopheles maculipennis</i> . <i>Riv. Malariol.</i> , <b>8</b> , pp. 393-400, 479.   |







## ON SEASONAL PREVALENCE OF ANOPHELES SPECIES IN SOUTH-EASTERN MADRAS\*.

BY

PAUL F. RUSSELL,

AND

T. RAMACHANDRA RAO.

[October 1, 1941]

### INTRODUCTION.

PUBLISHED observations regarding the seasonal distribution of anophelines in south-eastern Madras are meagre and sometimes misleading. This paper reports analyses of original data relating to one section of this area, over a period of three years (March 1937 to February 1940). It is based on continuous, routine and standardized collections of larvæ and adults made at the field station of Malaria Investigations, located at Pattukkottai in Tanjore District, where research is being conducted in the ecology of anophelines, and the epidemiology and experimental control of malaria. For other reports bearing on this paper *see* Rao and Russell (1940); Russell, Menon and Rao (1938); Russell and Rao (1940); Russell and Rao (1940a; 1940b); and Russell, Rao and Jacob (1938).

These studies have been limited to Pattukkottai taluk, which has an area of about 700 square miles, with uniform topographical and meteorological conditions. In marked contrast to the rich and compact alluvial soil of the deltaic (Cauvery River) section of Tanjore District, Pattukkottai taluk has organically poor, light, ferruginous soil (technically called sandy loam†). Prior

---

\* The studies on which this paper is based were conducted with the support, and under the auspices, of the International Health Division of the Rockefeller Foundation, co-operating with the King Institute of Preventive Medicine, Guindy, the Pasteur Institute of Southern India, Coonoor, and the Public Health Department of Madras Province.

† Geologically, most of Pattukkottai taluk appears to belong to the same series as the Cuddalore sand-stones of S. India, being covered over by laterite formation, gravel and sand.



to the inauguration of the Cauvery-Mettur Irrigation Project in 1933, it was a dry tract, with little rice or other wet crops. The north-east monsoon in November and December and a relatively few agricultural wells provided water. Since 1933, the country-side has been dominated by irrigation canals, which flood it with enormous quantities of the Cauvery water for eight months in the year. The studies reported here are concerned with conditions subsequent to the introduction of canal irrigation. Unfortunately, there are no pre-irrigation records.

### CLIMATE AND SEASON.

The Pattukkottai climate is dry and tropical. Table I gives monthly meteorological data. Temperature and relative humidities were measured continuously at the station by a recording thermo-humidograph\*. Weekly means were calculated from two-hourly means, read from the record. Therefore, the mean temperature and relative humidity figures, as presented in this paper, are true means and not merely the means of maximum and minimum figures. Records of daily maximum and minimum temperatures were taken from an ordinary Sixes thermometer. For some months records were also kept of the 4-00 p.m. relative humidity as measured by a whirling psychrometer. These observations indicated that weekly mean temperature and relative humidity figures reflected seasonal trends better than either the mean maxima or the 4-00 p.m. readings, and they have, therefore, been used in the tables. Saturation deficiency of the atmosphere, in grammes per cubic metre of air, which has sometimes been useful in studying insect bionomics, was calculated from mean temperature and relative humidity figures with the aid of Baker's Nomogram.

### TEMPERATURES.

Mean monthly temperatures ranged from 24.2°C. (75.6°F.) in December 1938 to 30.5°C. (86.9°F.) in June 1937 and 1939, and May 1938. December and January were the coolest months, while May and June were the hottest. Highest temperatures were recorded in 1937, on June 10, 11, and 12 at 37.8°C. (100°F.), in 1938, on May 27 at 38.3°C. (101°F.), and in 1939, on May 30 at 37.8°C. (100°F.). Lowest temperatures were recorded on December 7, 1937, at 17.8°C. (64°F.), on December 14 and 23, 1938, at 20°C. (68°F.), and on January 13, 14 and 15, 1940, at 18.9°C. (66°F.).

Pattukkottai is eight miles from the coast (Palk Strait) so that temperatures tended to be equable, daily ranges varying from 8° to 10°F. in the cool rainy months and 12° to 15°F., in the hotter dry months. Weekly records indicate that highest temperatures were usually recorded between 2 and 3 p.m., lowest at about 5-30 a.m.

### RELATIVE HUMIDITY AND SATURATION DEFICIENCY.

Highest mean monthly relative humidities were 81.7 per cent in November 1937, 80.9 per cent in December 1938, and 81.6 per cent in November 1939. Lowest mean monthly relative humidities were 61.8 per cent in June 1937, 60.8 per cent in June 1938, and 65.4 per cent in July 1939. Highest mean saturation deficiencies corresponded as follows : 11.8 grammes per cubic metre in June 1937,

\* Casella Model No. H620.



11.5 in June 1938, and 10.7 in July 1939. Lowest deficiencies were 4.1 in November 1937, 4.2 in December 1938, 4.6 in January, and 4.7 in November 1939. The mean relative humidity varied from about 82 per cent in wet months to about 60 per cent in dry months. This indicates a moderately humid climate throughout the year, with low points in June and July.

### RAINFALL.

The annual rainfall in Pattukkottai, based on figures for fifty years, averages 44.8 inches, with 54 rainy days. More than half falls in October, November and December, during the north-east monsoon. Unlike many other areas of India, the south-east coast of Madras has a scanty south-west monsoon, with a few sporadic showers from June to September. In some years, the area has more than normal pre-monsoon rain, the so-called 'mango showers', in April and May. In two of the three years under observation, there were heavy rains in April and May which modified mosquito breeding. Except in the monsoon months of October, November and December, rain generally falls in short heavy showers, the average number of inches per rainy day being highest in April and May, rather than in the monsoon season.

TABLE I.

*Meteorological data recorded at Pattukkottai, January 1937 to February 1940.*

Year and month.	MEANS.			RAINFALL.			Incoming irrigation water, Pattukkottai town. average cusecs.	REMARKS.
	Relative humidity, per cent.	Saturation deficiency, grammes per cubic metre.	Temperature, °C.	Inches	Rainy days.	Average inches per rainy day.		
January 1937	81.4	4.3	25.2	0.64	2	0.32	30.0	
February ..	81.2	4.7	27.2	0.30	2	0.15	20.0	
March ..	76.6	6.3	28.1	0.43	1	0.43	0.0	
April ..	78.1	6.2	29.0	7.28	4	1.82	0.0	
May ..	79.0	6.4	30.3	2.46	2	1.23	0.0	
June ..	61.8	11.8	30.5	0.44	3	0.15	56.7	
July ..	61.9	11.0	29.2	2.24	4	0.56	91.5	
August ..	66.3	9.5	29.4	10.06	9	1.12	80.0	
September ..	70.6	8.3	28.8	2.09	5	0.42	121.7	
October ..	80.9	5.0	27.5	3.06	9	0.34	95.3	
November ..	81.7	4.1	24.6	9.69	19	0.51	71.3	
December ..	75.2	4.4	24.9	0.21	2	0.11	69.3	
January 1938	75.5	5.8	25.8	0.89	4	0.22	60.2	
February ..	74.7	6.5	26.8	1.63	3	0.54	5.0	
March ..	78.4	5.7	28.3	2.76	6	0.46	0.0	
April ..	75.3	7.5	30.2	0.77	3	0.26	0.0	
May ..	70.1	9.3	30.5	1.56	4	0.39	0.0	
June ..	60.8	11.5	29.4	0.18	3	0.06	26.7	
July ..	64.5	10.5	29.5	2.20	7	0.31	108.3	
August ..	78.5	6.0	28.4	7.63	12	0.64	117.1	
September ..	74.5	7.2	28.5	3.39	4	0.85	115.1	



TABLE I—*concl.*

Year and month.	MEANS.			RAINFALL.			Incoming irrigation water, Pattukkottai town, average cusecs.	REMARKS.
	Relative humidity, per cent.	Saturation deficiency, grammes per cubic metre.	Temperature, °C.	Inches.	Rainy days.	Average inches per rainy day.		
October 1938	74.5	6.8	27.6	8.93*	11	0.81	123.6	*Taken from Taluk Office figure.
November ..	74.1	6.3	26.0	3.08	5	0.62	69.5	
December ..	80.9	4.2	24.2	3.27	9	0.36	57.5	
January 1939	79.9	4.6	24.5	2.01	5	0.40	34.9	
February ..	74.0	6.5	26.1	0.00	0	0.00	7.2	
March ..	73.9	6.9	27.6	2.30	1	2.30	0.0	
April ..	77.3	6.5	28.7	9.26	6	1.54	0.0	
May ..	73.5	7.6	29.2	5.13	3	1.71	0.0	
June ..	68.2	9.8	30.5	1.23	5	0.25	34.3	
July ..	65.4	10.7	30.4	2.90	6	0.48	99.0	
August ..	67.9	9.7	29.8	3.88	3	1.29	131.9	Cyclone.
September ..	74.7	7.6	29.9	2.61	8	0.33	141.9	
October ..	80.9	5.3	28.5	10.33	25	0.41	104.3	
November ..	81.6	4.7	26.4	18.98	15	1.27	63.8	
December ..	75.2	6.2	26.4	0.24	5	0.05	80.5	
January 1940	73.9	6.3	25.8	0.00	0	0.00	79.5	
February ..	71.0	7.7	27.5	0.00	0	0.00	0.0	

Notes.—(1) The above data were gathered on the basis of weeks, a month being made up of all weeks commencing in that month. Hence some months include four and others five weeks in the table.

(2) It must be noted that the cusecs of incoming water refer to water flowing past Pattukkottai town only and not over the whole taluk. Therefore, our figures constitute an index.

(3) The rainfall figures from January 1937 to February 1938 were taken from Taluk Office records, while from March 1938 onwards they were taken from the records of the Field Station rain gauge.

Occasionally, this area experiences severe cyclones, coming in from the Bay of Bengal, and causing considerable damage. On November 16, 1939, there was such a storm, marked by 8.3 inches of rain in 24 hours. The previous cyclone occurred in November 1935.

#### IRRIGATION WATER.

Irrigation seasons commenced in mid-June and terminated at the end of January, except in 1937-38, when water continued to flow in the canals for some days in February. In 1937-38 there was a continuous supply throughout the season, but in 1938-39 and 1939-40, beginning in late October, there was a somewhat irregular 'turn system', each branch canal being closed for a few days in turn. In the 1939-40 season there was a complete breakdown after the cyclone, so that for 25 days there was no flow.

Table I gives average number of cusecs per day of irrigation water which entered the Rajamatam canal, contiguous to Pattukkottai town. This is one



branch of the system, and the cusec figures should not be confused with those relating to the total irrigation water entering the area. These figures furnish an index, showing seasonal trends and variations in amount of actual irrigation.

## SEASONS.

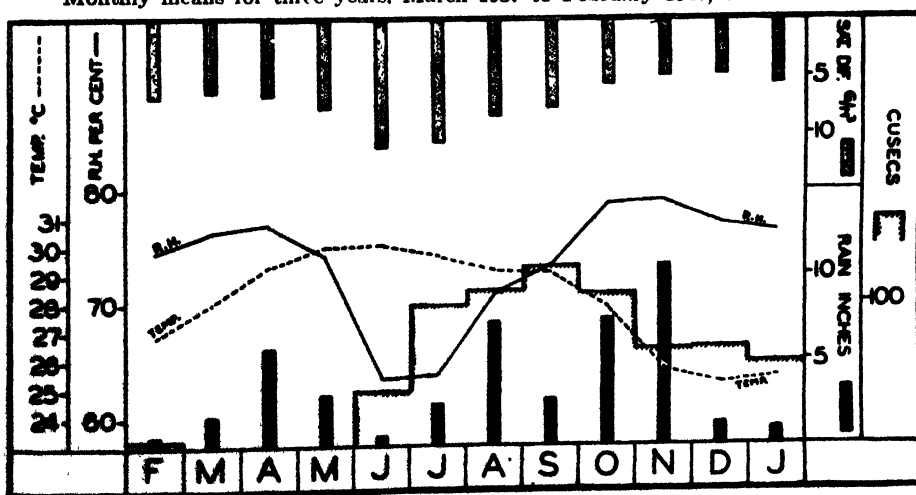
It seems logical to divide the Pattukkottai year into six seasons, as follows:—

Months.	Irrigation.	Climate.	Anopheles abundance.	Malaria.
Feb.-Mar. ..	Early non-irrigation.	Cool—Dry	Moderate	Non-malaria season.
Apr.-May \ ..	Late non-irrigation.	Hot—Dry	Scanty	
June-July ..	First quarter irrigation.	Hot—Dry	Increasing	
Aug.-Sep. ..	Second quarter irrigation.	Hot—Dry	Greatest abundance.	Malaria season.
Oct.-Nov. ..	Third quarter irrigation.	Hot—Wet	Declining	
Dec.-Jan. ..	Last quarter irrigation.	Coolest—Wet	Moderate	

Table II gives the average meteorological data and cusecs of irrigation water, by months and seasons. (Chart 1 shows graphically the same data by months.

CHART 1.  
METEOROLOGICAL DATA.

Monthly means for three years. March 1937 to February 1940, Pattukkottai.





Text-figure 1.

Card for recording and analysing data regarding collections of *Anopheles* larvæ, Pattukkottai (obverse).

56		55 M		54 S		53 C		52 E		51 L		50 N		49 O		48 S		47	
1		2		3		4		5		6		7		8		9		10	
11		12		13		14		15		16		17		18		19		20	
21		22		23		24		25		26		27		28		29		30	
31		32		33		34		35		36		37		38		39		40	
41		42		43		44		45		46		47		48		49		50	
51		52		53		54		55		56		57		58		59		60	
Number.		Date		Malaria Investigations Collections of Larvæ		GENERAL CHARACTER OF BREEDING PLACE		PASTEUR INSTITUTE, COONCOOR, PATTUKKOTTAI FIELD STATION		Region		Time Spent Dipping		Number of Dips					
Breeding Place		Water		Area Dipped															
Temp. Air		Remarks																	
1		2		3		4		5		6		7		8		9		10	
11		12		13		14		15		16		17		18		19		20	
21		22		23		24		25		26		27		28		29		30	
21		22		23		24		25		26		27		28		29		30	
21		22		23		24		25		26		27		28		29		30	
21		22		23		24		25		26		27		28		29		30	
21		22		23		24		25		26		27		28		29		30	
21		22		23		24		25		26		27		28		29		30	
21		22		23		24		25		26		27		28		29		30	
21		22		23		24		25		26		27		28		29		30	
21		22		23		24		25		26		27		28		29		30	
21		22		23		24		25		26		27		28		29		30	
21		22		23		24		25		26		27		28		29		30	
21		22		23		24		25		26		27		28		29		30	
21		22		23		24		25		26		27		28		29		30	
21		22		23		24		25		26		27		28		29		30	
21		22		23		24		25		26		27		28		29		30	
21		22		23		24		25		26		27		28		29		30	
21		22		23		24		25		26		27		28		29		30	
21		22		23		24		25		26		27		28		29		30	
21		22		23		24		25		26		27		28		29		30	
21		22		23		24		25		26		27		28		29		30	
21		22		23		24		25		26		27		28		29		30	
21		22		23		24		25		26		27		28		29		30	
21		22		23		24		25		26		27		28		29		30	
21		22		23		24		25		26		27		28		29		30	
21		22		23		24		25		26		27		28		29		30	
21		22		23		24		25		26		27		28		29		30	
21		22		23		24		25		26		27		28		29		30	
21		22		23		24		25		26		27		28		29		30	
21		22		23		24		25		26		27		28		29		30	
21		22		23		24		25		26		27		28		29		30	
21		22		23		24		25		26		27		28		29		30	
21		22		23		24		25		26		27		28		29		30	
21		22		23		24		25		26		27		28		29		30	
21		22		23		24		25		26		27		28		29		30	
21		22		23		24		25		26		27		28		29		30	
21		22		23		24		25		26		27		28		29		30	
21		22		23		24		25		26		27		28		29		30	
21		22		23		24		25		26		27		28		29		30	
21		22		23		24		25		26		27		28		29		30	
21		22		23		24		25		26		27		28		29		30	
21		22		23		24		25		26		27		28		29		30	
21		22		23		24		25		26		27		28		29		30	
21		22		23		24		25		26		27		28		29		30	
21		22		23		24		25		26		27		28		29		30	
21		22		23		24		25		26		27		28		29		30	
21		22		23		24		25		26		27		28		29		30	
21		22		23		24		25		26		27		28		29		30	
21		22		23		24		25		26		27		28		29		30	
21		22		23		24		25		26		27		28		29		30	
21		22		23		24		25		26		27		28		29		30	
21		22		23		24		25		26		27		28		29		30	
21		22		23		24		25		26		27		28		29		30	
21		22		23		24		25		26		27		28		29		30	
21		22		23		24		25		26		27		28		29		30	
21		22		23		24		25		26		27		28		29		30	
21		22		23		24		25		26		27		28		29		30	
21		22		23		24		25		26		27		28		29		30	
21		22		23		24		25		26		27		28		29		30	
21		22		23		24		25		26		27		28		29		30	
21		22		23		24		25		26		27		28		29		30	
21		22		23		24		25		26		27		28		29		30	
21		22		23		24		25		26		27		28		29		30	
21		22		23		24		25		26		27		28		29		30	
21		22		23		24		25		26		27		28		29		30	
21		22		23		24		25		26		27		28		29		30	
21		22		23		24		25		26		27		28		29		30	
21		22		23		24		25		26		27		28		29		30	
21		22		23		24		25		26		27		28		29		30	
21		22		23		24		25		26		27		28		29		30	
21		22		23		24		25		26		27		28		29		30	
21		22		23		24		25		26		27		28		29		30	
21		22		23		24		25		26		27		28		29		30	
21		22		23		24		25		26		27		28		29		30	
21		22		23		24		25		26		27		28		29		30	
21		22		23		24		25		26		27		28		29		30	
21		22		23		24		25		26		27		28		29		30	
21		22		23		24		25		26		27		28		29		30	
21		22		23		24		25		26		27		28		29		30	
21		22		23		24		25		26		27		28		29		30	
21		22		23		24		25		26		27		28		29		30	
21		22		23		24		25		26		27		28		29		30	
21		22		23		24		25		26		27		28		29		30	
21		22		23		24		25		26		27		28		29		30	
21		22		23		24		25		26		27		28		29		30	
21		22		23		24		25		26		27		28		29		30	
21		22		23		24		25		26		27		28		29		30	
21		22		23		24		25		26		27		28		29		30	
21		22		23		24		25		26		27		28		29		30	
21		22		23		24		25		26		27		28		29		30	
21		22		23		24		25		26		27		28		29		30	
21		22		23		24		25		26		27		28		29		30	
21		22		23		24		25		26		27		28		29		30	
21		22																	



Text-figure 2.

Card for recording and analysing data regarding collections of *Anopheles* larvæ, Pattukkottai (reverse).

No.	SPECIES.	IDENTIFICATIONS.				
		Larvæ.	Adults Hatched out		Pupæ caught.	TOTAL.
			M.	F.		
22	Aconitus					
23	Annularis					
24	Barbirostris					
25	Culicifacies					
26	Hyrceanus v. nigerrimus					
27	Jamesi					
28	Pallidus					
29	Stephensi					
30	Subpictus					
31	Tessellatus					
32	Vagus					
33	Varuna					
34						
35						
	Total identified					
	Unidentified					
	Total Collected					
36	Culicines					

REMARKS

22 23 24 25 26 27 28



TABLE II.

Means of meteorological data recorded at Pattukkottai Field Station, March 1937 to February 1940.

Month.	MONTHLY MEANS.					SEASONAL MEANS.				
	Tempera- ture, °C.	Relative humidity, per cent.	Saturation deficiency, gm. cm.*	Rainfall, inches.	Cusecs of incoming irrigation water.	Tempera- ture, °C.	Relative humidity, per cent.	Saturation deficiency, gm. cm.*	Rainfall, inches.	Cusecs of incoming irrigation water.
February ..	26.8	74.5	6.9	0.54	4.1	27.4	75.4	6.6	2.37	2.2
March ..	28.0	76.3	6.3	1.83	0.0	29.7	75.6	7.3	8.82	0.0
April ..	29.3	76.9	6.7	5.77	0.0					
May ..	30.0	74.2	7.8	3.05	0.0	29.9	63.7	10.9	3.07	69.4
June ..	30.1	63.5	11.1	0.62	39.2					
July ..	29.7	63.9	10.7	2.45	99.6	29.2	72.1	8.1	9.89	118.0
August ..	29.2	70.9	8.4	7.19	109.7					
September ..	29.1	73.3	7.7	2.70	126.2	26.8	79.0	5.4	18.02	88.0*
October ..	27.9	78.8	5.7	7.44	107.7					
November ..	25.7	79.1	5.0	10.53	68.2	25.3	76.8	5.3	2.21	63.7
December ..	25.2	77.1	4.9	1.24	69.1					
January ..	25.4	76.4	5.6	0.97	58.2					

\* gm. cm. = Saturation deficiency in grammes per cubic metre.

Note.—See footnotes in Table I.



## GENERAL CONSIDERATIONS AND METHODS.

The seasonal abundance of a mosquito species may depend to a large extent upon prevalence of preferred habitat. However favourable meteorological conditions may be, if preferred habitats are absent, a species cannot become abundant. On the other hand, if the preferred habitat predominates when other factors are unfavourable, the net result is not predictable. There may be low incidence or the habitat factor may mask seasonal influences so that incidence is high. It is, therefore, important, in studying seasonal prevalences of mosquito populations, to keep both seasonal and habitat factors clearly in view. An understanding of seasonal prevalences requires a study of both larval and adult densities.

A carefully planned larval survey is the first requisite, with routine visits to the same types of habitat and breeding places. Fluctuations in intensity of breeding in individual places will reveal periods of active breeding and may indicate optimum seasons. But, since the total output of adults in a given area depends largely on the extent and abundance of suitable breeding places, it is also necessary to keep in mind seasonal fluctuations in occurrence of the habitat itself.

Adult surveys should bring out the relative abundance of a species, season by season. A survey that fails to consider specific resting habits will not obtain a correct picture of seasonal prevalence, for it is well known that species differ in their preferences. Theoretically, a period of larval abundance is followed by one of adult abundance. A carefully planned survey, taking into consideration preferences in larval habitats and adult resting places, will generally show related curves. However, in practice, the postulated theoretical agreement may not be seen, owing to limitations of collection and observation and to seasonal factors.

Density indices require more attention, since entomologists do not yet possess exact methods of measurement. Intensive observations in this regard will be reported later. The following methods were used for the studies here reported.

Routine larval catching stations, embracing all types of habitat, were established. General and widespread collections were made, taking into account distribution and abundance of breeding places, so that each type of habitat received attention roughly proportional to its importance. Standardized technique and dippers of uniform design were employed. The actual time spent in dipping was noted on a special survey card, used for field and laboratory records and for analysis of data (Text-figures 1, 2). There was no copying of records.

A number of routine adult collection stations were selected. These were dwellings for humans, or animals, or both. Routine practice required thirty-minute collections by experienced assistants, using standard equipment consisting of a suction tube, head light, and test-tubes as containers. All figures were reduced to number collected per man-hour. This appeared to be a safe guide to relative density for those mosquitoes that prefer dwellings as usual daytime resting places.

Traps of special design were set up in certain places to attract those mosquitoes that do not rest normally in dwellings. There were also miscellaneous and general collections to substantiate information gathered from routine stations and traps and to seek information about rarer species.



Most dwellings in this area were thatched, mud-walled structures, so that the catching stations were all of the same type. Six to eight such stations were established in a village. Plate XXX, figures 1 and 2 show typical adult collection stations.

Plate XXXI, figure 3 shows a portable stable trap which was attractive to *A. hyrcanus*, a species uncommon in routine collections in dwellings. The trap was built to specifications given by Magoon (1935). It was used in five locations, some miles apart, and was usually baited with a calf. When goats were used, fewer anophelines were attracted. Human bait was offered several times, but on such occasions not more than a dozen anophelines entered the trap. The usual practice was to introduce a calf at 6-00 p.m. and close the door. The fish-trap entrances were plugged with paper at 6-00 the next morning, the calf was removed, and collections made. The trap attracted and retained large numbers of female, but very few male, *A. hyrcanus*. The data of collections in this trap are shown in Table VII.

### OBSERVATIONS.

Data gathered for twelve species of *Anopheles* are presented in Tables III to XI. Table III gives the total number of larvæ collected in each calendar month, all years combined, from March 1937 to February 1940, and also shows the number of times each species was taken. This table gives a fair idea of the prevalence of larvæ by species and month. But, to emphasize intensity and relative frequency, Table IV has been prepared. This gives figures for the average number of larvæ per 10 minutes of standardized dipping for each species, in each month during the period June 1937 to February 1940, taking all positive collections into consideration, whether a given species was found or not.

Table V presents the total number of adults of each species collected during the period, the same months of all years being combined. This table gives figures for all collections, and presents a fair idea of seasonal prevalences. But, since the total time spent in collecting varied somewhat from month to month, figures are given in Table VI to show prevalence by months in mosquitoes per man-hour, calculated from standardized collections made in routine catching stations. These two tables give ample information regarding prevalence of adults of those anophelines that resort to dwellings during daytime. But they do not disclose true prevalence of such species as *A. hyrcanus*. As will be described below, this species definitely avoids dwellings in the daytime. The prevalence of *A. hyrcanus* is reflected better by collections in the portable stable trap, as shown in Table VII.

Each species may be considered individually.

#### *A. aconitus* (Chart 2).

This was a rare species, for only one larva and 72 adults were collected during the whole period of study. In spite of these meagre collections, a definite seasonal trend was apparent, for adults were taken only between January and April, February and March being the months of greatest prevalence.

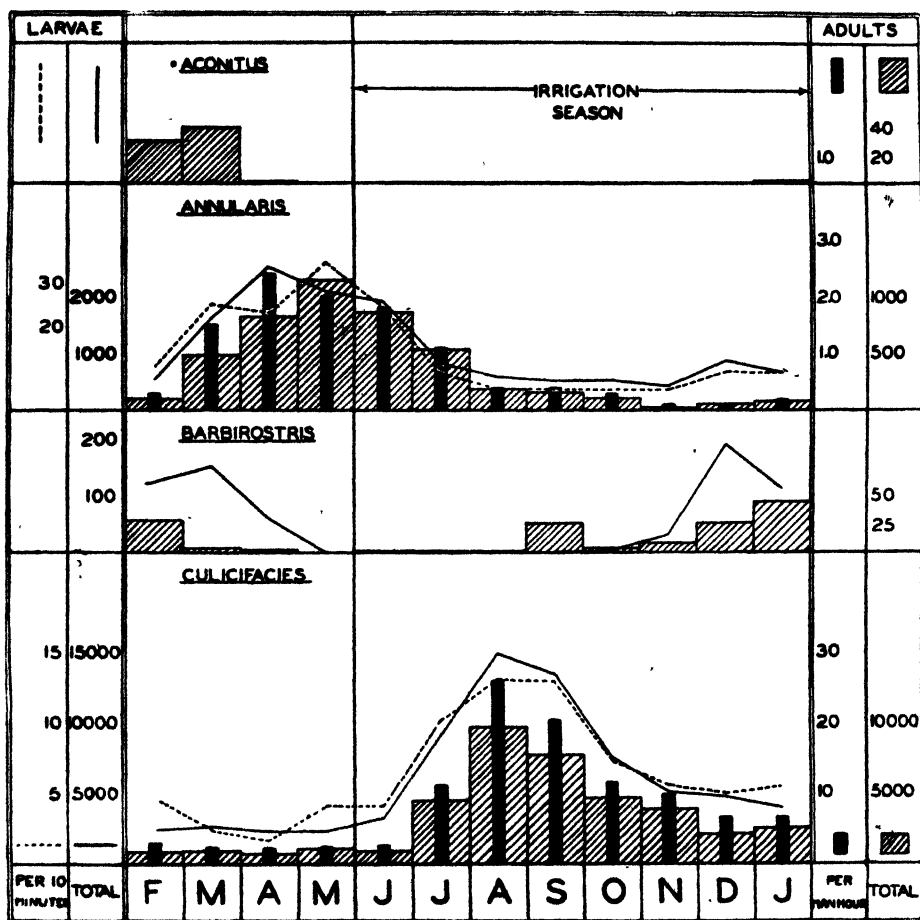
#### *A. annularis* (Chart 2).

This was a fairly common species and occurred throughout the year. It showed a marked seasonal prevalence, being abundant from March to July, the



CHART 2.

Seasonal prevalence of *A. aconitus*, *A. annularis*, *A. barbirostris* and *A. culicifacies* as shown by collections of larvæ and adults.



greatest numbers being recorded in April and May. The total larvæ collected and adult per man-hour figures indicate that April was the most suitable month, although the peak shifted to May in the curve for total adults collected. This species showed a marked preference for tanks, a habitat that exists throughout the year, so that a maximum prevalence during April and May indicated a definite seasonal trend.

While it is true that tank waters are diluted from July or August onwards by large volumes of irrigation water, this does not appear to explain the decline in numbers of *A. annularis*, because this downward trend began in June, when the tanks contained the least water. Further, there was no marked increase or reduction of vegetation in the tanks from May onwards to account for the decline. Therefore, it seems that the greater abundance of *A. annularis* in April and May was an effect of seasonal factors, beginning in March.



TABLE III.

Total positive collections of anopheline larvæ listed by species and months, March 1937 to February 1940, all years combined.

Month.	annularis.		barbirostris.		culicifacies.		hyrcamus.		jamesi.		pallidus.		stephensi.		subpictus.		tessellatus.		vagus.		serrana.	
	Total larvæ col-lected.	Times col-lected.	Total larvæ col-lected.	Times col-lected.	Total larvæ col-lected.	Times col-lected.	Total larvæ col-lected.	Times col-lected.	Total larvæ col-lected.	Times col-lected.	Total larvæ col-lected.	Times col-lected.	Total larvæ col-lected.	Times col-lected.	Total larvæ col-lected.	Times col-lected.	Total larvæ col-lected.	Times col-lected.	Total larvæ col-lected.	Times col-lected.	Total larvæ col-lected.	Times col-lected.
January ..	676	68	114	34	3,958	279	2,136	195	300	56	529	84	231	5	1,159	156	4	3	387	72	1,015	76
February ..	534	72	125	32	2,428	248	506	79	248	54	315	56	306	4	1,526	152	0	..	604	107	965	55
March ..	1,615	131	152	14	2,623	66	17	7	558	100	560	70	303	8	5,118	273	0	..	1,862	190	782	65
April ..	2,523	135	62	5	2,355	117	1	1	432	74	812	107	535	12	4,679	243	0	..	1,357	155	1,445	66
May ..	2,015	162	0	0	2,374	178	44	15	255	85	1,010	123	546	3	4,726	326	0	..	654	110	2,987	115
June ..	1,913	133	1	1	3,286	264	1	1	253	63	467	74	405	3	7,420	379	0	..	110	29	4,127	123
July ..	834	87	1	1	9,121	479	20	5	107	23	616	69	97	6	8,088	373	0	..	78	12	3,077	107
August ..	590	69	1	1	14,865	655	199	37	75	17	1,115	100	346	7	7,652	438	0	..	337	55	3,338	110
September ..	637	53	1	1	13,422	679	470	82	143	27	2,677	238	433	4	4,671	389	1	1	573	89	3,218	106
October ..	521	70	4	4	7,574	583	1,734	117	71	15	2,299	311	312	5	4,685	378	0	..	425	98	2,408	96
November ..	439	36	32	14	5,141	437	1,660	199	28	10	1,373	205	334	6	6,149	410	5	5	959	179	2,046	114
December ..	861	74	190	31	4,794	417	2,517	275	159	24	932	162	132	4	3,610	302	14	9	1,046	145	2,400	117
Total ..	13,078	..	683	..	71,941	..	9,305	..	2,629	..	12,705	..	3,980	..	59,483	..	24	..	8,392	..	27,828	..

Note.—The total larvæ collected refer to all collections in all months.



TABLE IV.

Average number of larvæ of each species collected, per 10 minutes of actual dipping, in all breeding places showing *Anopheles* larvæ, June 1937 to February 1940, same months of all years combined.

Month.	<i>annularis</i> .	<i>barbivrosus</i> .	<i>culicifacies</i> .	<i>hyrcanus</i> .	<i>jamesi</i> .	<i>pallidus</i> .	<i>stephensi</i> .	<i>subpictus</i> .	<i>tessellatus</i> .	<i>vagus</i> .	<i>varuna</i> .	Total minutes spent dipping.
January ..	0.9	0.2	5.5	3.0	0.4	0.7	0.3	1.6	*	0.5	1.4	7,184
February ..	1.0	0.2	4.7	1.0	0.5	0.6	0.6	2.9	0.0	1.2	1.9	5,210
March ..	2.5	0.1	2.4	*	1.0	0.9	0.6	8.2	0.0	2.6	1.5	5,120
April ..	2.3	0.2	1.7	*	0.8	1.7	1.3	7.7	0.0	2.8	3.5	4,113
May ..	3.5	0.0	4.1	0.1	0.5	1.8	1.0	6.9	0.0	1.1	5.7	5,218
June ..	2.4	*	4.1	*	0.3	0.6	0.6	9.7	0.0	0.1	5.9	7,037
July ..	0.9	*	10.1	*	0.1	0.7	0.1	8.9	0.0	0.1	3.4	9,075
August ..	0.5	*	13.1	0.2	0.1	1.0	0.3	6.7	0.0	0.3	2.9	11,424
September ..	0.5	*	12.9	0.5	0.1	2.6	0.4	4.5	*	0.6	3.1	10,417
October ..	0.5	*	7.3	1.7	0.1	2.2	0.3	4.5	0.0	0.4	2.3	10,369
November ..	0.5	*	5.6	1.8	*	1.5	0.4	6.7	*	1.0	2.2	9,198
December ..	0.9	0.2	5.0	2.6	0.2	1.0	0.1	3.7	*	1.1	2.5	9,667

\* = Less than 0.1.



TABLE V.

Total routine and general collections of anopheline adults listed by months and species for three years, March 1937 to February 1940, all years combined.

Month.	<i>aconitus.</i>	<i>annularis.</i>	<i>barbros- tria.</i>	<i>culici- facies.</i>	<i>hyrcanus.</i>	<i>jamesi.</i>	<i>pallidus.</i>	<i>stephensi.</i>	sub- <i>pictus</i> females only.	<i>tessella- tus.</i>	<i>vagus</i> females only.	<i>vagus</i> or sub- <i>pictus</i> males only.	<i>varuna.</i>	Total.
January ..	1	85	45	2,560	2,003	245	174	2	2,474	84	2,819	3,179	154	13,825
February ..	30	106	28	960	89	155	12	3	1,337	37	2,177	1,794	91	6,819
March ..	40	492	4	982	8	196	16	1	2,221	5	3,884	2,798	92	10,739
April ..	1	803	2	791	0	114	17	1	2,516	1	2,513	2,083	140	8,982
May ..	0	1,151	0	1,167	1	118	13	0	5,390	0	2,491	2,429	198	12,958
June ..	0	881	1	983	2	38	10	1	3,486	0	348	1,188	180	7,118
July ..	0	535	1	4,524	10	98	50	1	11,253	3	597	5,974	132	23,178
August ..	0	189	1	9,700	19	96	87	0	25,940	1	2,480	16,337	120	54,970
September..	0	156	26	7,777	873	37	154	0	24,074	1	3,654	17,798	81	54,631
October ..	0	111	4	4,670	1,206	15	112	0	23,098	3	4,999	14,618	111	48,947
November ..	0	25	9	3,905	1,937	16	73	2	13,066	6	4,162	7,638	215	31,054
December ..	0	53	27	2,165	1,113	27	56	0	3,120	37	2,149	2,438	185	11,370
Total. ..	72	4,587	148	40,184	7,261	1,155	774	11	117,975	178	32,273	78,274	1,699	284,591



TABLE VI.

*Routine adult anopheline collections, per man-hour, by months and species, collected in Pattukkottai taluk and town, March 1937 to February 1940, all years combined.*

Month.	Total hours spent collecting.	aco-nitus.	annu-laris.	barbi-rostris.	culici-facies.	hyr-canus.	jamest. pallidus.	stephensi.	sub-pictus females only.	tessel-latus.	vagus females only.	vagus or sub-pictus males only.	varuna.	Total.
January ..	332.0	†	0.2	*	6.7	0.3	0.6	0.2	*	5.7	7.0	8.4	0.4	29.7
February ..	258.0	0.1	0.3	*	2.8	0.1	0.4	*	*	3.1	5.2	4.6	0.3	17.0
March ..	203.5	*	1.5	*	2.4	*	0.6	0.1	†	5.1	8.8	6.3	0.3	25.1
April ..	223.0	*	2.4	†	2.3	†	0.3	0.1	*	6.6	8.0	6.5	0.5	26.7
May ..	311.0	†	2.1	†	2.4	†	0.3	*	†	8.7	4.0	5.6	0.5	23.6
June ..	273.5	†	1.8	†	2.3	†	0.1	*	*	7.2	0.9	3.3	0.6	16.2
July ..	311.5	†	1.1	†	11.0	*	0.2	0.1	*	20.3	1.5	14.6	0.4	49.2
August ..	293.5	†	0.4	†	25.7	*	0.3	0.2	†	50.6	6.1	40.2	0.3	123.8
September ..	259.0	†	0.4	*	20.3	†	0.1	0.2	†	46.5	10.9	43.3	0.2	121.9
October ..	317.5	†	0.3	†	11.6	*	*	0.1	†	51.6	13.2	38.3	0.3	115.4
November ..	270.0	†	0.1	†	9.9	0.1	*	0.1	*	27.1	10.6	16.3	0.6	64.8
December ..	258.0	†	0.1	*	6.7	0.1	0.1	0.1	†	8.5	6.1	7.6	0.6	30.0

Notes.—\* indicates specimens collected, but less than 0.1 per man-hour.

† indicates specimens not collected.



TABLE VII.

Portable stable trap collections, by months and species, 24 months, February 1938 to January 1940.

Month.	Num- ber of collec- tions.	<i>annularis</i> .		<i>barbitrostris</i> .		<i>culicifacies</i> .		<i>hyrcanus</i> .		<i>jamesi</i> .		<i>pallidus</i> .		sub- <i>pictus</i> females only.	<i>vagus</i> females only.	<i>vagus</i> or sub- <i>pictus</i> males only.	<i>tesellatus</i> .		<i>varuna</i> .	
		Male.	Total.	Male.	Total.	Male.	Total.	Male.	Total.	Male.	Total.	Male.	Total.				Male.	Total.	Male.	Total.
January ..	28	0	9	0	18	1	36	0	1,402	0	5	0	103	182	121	0	0	5	0	8
February ..	16	0	0	0	3	1	1	0	41	0	1	0	0	6	7	0	0	0	0	1
March ..	18	0	6	0	3	2	9	0	1	0	2	0	0	212	152	9	0	0	0	2
April ..	32	0	1	0	2	0	1	0	0	0	0	0	1	203	38	11	0	0	0	0
May ..	45	0	22	0	0	0	29	0	1	1	10	0	2	1,220	581	11	0	0	0	4
June ..	38	0	15	0	1	0	14	0	2	0	2	0	4	634	23	4	0	0	0	3
July ..	45	0	19	0	1	1	42	0	7	0	0	0	5	2,055	38	18	0	0	0	4
August ..	39	0	5	0	1	4	149	0	14	0	0	0	17	5,756	216	22	0	0	0	18
September ..	21	0	8	0	0	4	184	0	550	0	2	0	45	4,362	95	24	0	0	0	2
October ..	26	0	2	0	0	0	20	0	843	0	0	0	49	2,237	91	18	0	0	0	1
November ..	18	0	0	0	0	0	16	0	1,671	0	1	0	11	207	30	1	0	0	0	2
December ..	24	0	6	0	4	0	24	0	807	0	0	1	19	107	85	0	0	3	0	11
Total ..	350	0	93	0	33	13	525	0	5,339	1	23	1	256	17,181	1,477	118	0	8	0	56

Note.—During this period the trap was baited with a calf in most of the collections, except in January 1940 when a goat was baited alternately with a calf. Occasionally, human bait was offered in the trap without success in attracting mosquitoes.

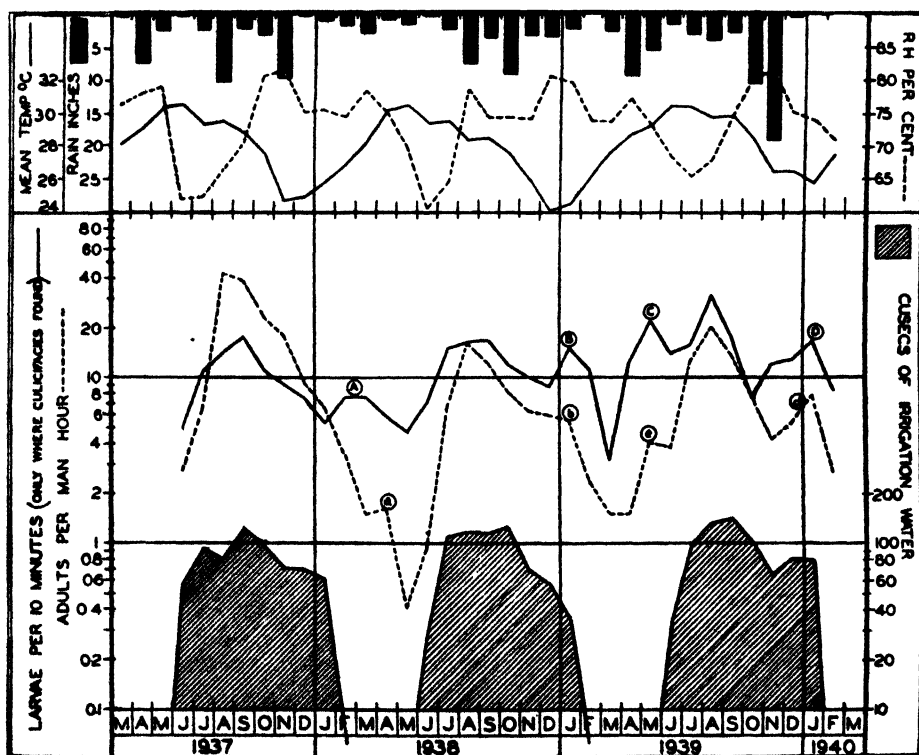


*A. barbirostris* (Chart 2).

This was an uncommon species, only 683 larvæ and 148 adults being taken. Larvæ and adults were most abundant between November and March, but it is questionable whether this was due to season or to predominance of favourable habitat. *A. barbirostris* preferred water organically rich with putrefying vegetation. Between November and March, towards and just after the end of irrigation, the area abounds in borrowpits and ditches containing stagnant water, full of decomposing organic material, which may explain the relative abundance of *A. barbirostris* at this time. There was no evidence that meteorological factors influenced its abundance in the cool season.

## CHART 3.

Seasonal prevalence of *A. culicifacies* in relation to conditions of climate and irrigation.

*A. culicifacies* (Charts 2 and 3).

This was the most important species, because of its abundance and because it was the chief malaria vector. It occurred throughout the year but showed definite seasonal trends. Tables III to VII show that this species was most abundant from July to October. Chart 2 presents the data graphically. It is apparent that the lowest points in the curves occurred from April to early June. This was a time when most breeding places of *A. culicifacies* were dry. The curves consistently show a marked and steady rise



for some weeks after irrigation water began to flow in mid-June. In view of the importance of this species, data concerning it are presented in further detail in Tables VIII and IX and in Chart 3. Seasonal trends appeared to be as follows:—

*June-July.*—There was a rapid rise in abundance of *A. culicifacies* larvæ and adults from mid-June onwards, i.e., from the beginning of the irrigation season. No correlation is evident between this rise and temperature, relative humidity, saturation deficiency or rainfall. It seems apparent that the marked upward trend in June and July was due mainly to a great increase in the number of favourable breeding places, such as irrigation canals and channels, seepage borrowpits, wet fallow ricefields and ditches. Chart 3 indicates that the rise commenced when relative humidity was lowest and theoretically most adverse.

As regards intensity of breeding, June and July were favourable months, and there was an enormous output of adults at this period. The great increase in these months must be attributed to a tremendous increase in number of ideal breeding places.

It is not clear how adult populations were affected by meteorological conditions during this period. Some authors have emphasized that a decrease in number of adults generally follows a period of high saturation deficiency. Hicks and Majid (1937), for instance, have shown in the Punjab a significant correlation between number of adults of *A. culicifacies* and variations in saturation deficiency, a rise in saturation deficiency being followed after one or two weeks by a fall in collections. It is of interest, therefore, that in Pattukkottai, a notable rise in the number of adults of this species commenced in June, when saturation deficiency was highest and continued in July when the deficiency was almost as great. The effect of high saturation deficiency is presumably on the longevity of adults. In Pattukkottai, the rate of increase in output of adults from the increasing number of favourable breeding places was apparently so great that it masked any adverse effect which might have come from a high saturation deficiency.

It may also be noted that no marked rise in relative humidity or fall in saturation deficiency was noticed as a result of the advent of large quantities of irrigation water which flooded the country-side. The higher humidity of later months of the year seemed to be due primarily to rainfall.

*August-September.*—These two months were peak months for larvæ and adults of *A. culicifacies*. The abundance of this species in these months seemed to be due directly to a maximum prevalence of favourable breeding places. The period coincided with the maximum flow of water in the canals (Table II). The intensity of prevalence of larvæ appeared to be at its peak during this season. In Chart 3, the curve for larvæ is plotted on the basis of average number collected per 10 minutes of actual collection, in places where *A. culicifacies* larvæ were taken. It is evident that in all three years, August or September showed the highest density.

August was the month of maximum prevalence of adult *A. culicifacies* in all three years. One might infer, therefore, that meteorological and breeding conditions established a balance each year at that time. For August, the average temperature was 29.1°C. (84.4°F.), average relative humidity 70.9 per cent, average saturation deficiency 8.4 grammes per cubic metre and average rainfall 7.19 inches.



TABLE VIII.

*A. culicifacies* larvæ and adults, collection data by months in Pattukkottai town and taluk, June 1937 to February 1940.

Year and month.	LARVÆ.			ADULTS.		
	Total <i>culicifacies</i> larvæ collected.	<i>A. culicifacies</i> larvæ per 10 minutes.		<i>A. culicifacies</i> adults.		
		All positive collections.	Only where <i>culicifacies</i> found.	Routine collections.		Total—all collections.
				Total collected.	Average per man-hour.	
June 1937 ..	367	3.1	5.0	146	2.7	244
July ..	1,052	5.4	10.8	1,025	6.4	1,402
August ..	3,379	9.1	14.1	3,812	42.6	4,084
September ..	3,744	12.0	17.6	2,775	38.3	3,394
October ..	2,087	7.0	11.0	1,745	23.4	2,474
November ..	1,305	5.4	9.0	1,809	17.9	2,167
December ..	1,422	4.0	7.5	685	9.3	812
January 1938 ..	700	2.7	5.3	703	6.5	840
February ..	1,372	4.7	7.6	280	3.4	392
March ..	1,171	3.0	7.6	134	1.5	247
April ..	490	1.7	5.8	151	1.6	162
May ..	312	1.0	4.7	55	0.4	62
June ..	841	2.2	7.1	81	0.9	91
July ..	6,238	11.3	15.9	767	6.8	850
August ..	6,751	12.6	16.5	1,724	16.1	2,081
September ..	5,983	13.1	16.8	978	12.4	2,175
October ..	3,829	7.9	12.0	843	8.2	993
November ..	2,143	4.9	10.0	462	6.3	801
December ..	1,610	4.6	8.7	442	5.9	702
January 1939 ..	1,353	5.1	15.2	563	5.5	659
February ..	562	4.9	11.3	192	2.3	274
March ..	83	0.7	3.2	125	1.5	193
April ..	207	1.6	12.2	126	1.5	187



TABLE VIII—concl'd.

Year and month.	LARVÆ.			ADULTS.		
	Total <i>culicifacies</i> larvæ collected.	<i>A. culicifacies</i> larvæ per 10 minutes.		<i>A. culicifacies</i> adults.		
		All positive collections.	Only where <i>culicifacies</i> found.	Routine collections.		Total— all collections.
				Total collected.	Average per man-hour.	
May ..	1,813	8.6	22.4	441	4.1	663
June ..	1,672	8.2	14.2	390	3.8	648
July ..	1,831	11.3	15.8	1,735	12.7	2,272
August ..	4,735	20.1	31.5	1,994	20.6	3,535
September ..	3,695	13.6	18.0	1,517	14.1	2,208
October ..	1,658	4.6	7.5	1,095	7.8	1,203
November ..	1,693	7.1	12.2	413	4.3	937
December ..	1,762	6.8	12.9	591	5.4	651
January 1940 ..	1,905	9.8	16.7	965	7.9	1,061
February ..	494	4.3	8.5	250	2.7	294

*October–November.*—During these months there was a definite decline in numbers of adults and larvæ of *A. culicifacies*. There did not appear to be any reduction in the total area of suitable water surface available for breeding. All breeding places holding irrigation water continued to be full, but, for some reason, both intensity of breeding and density of adults sharply declined. This was in clear contrast to the months of June and July, when there was an upward trend, apparently due to an enormous increase in breeding places, and when relative humidity was low and saturation deficiency high. In October–November, the downward trend was not correlated with any reduction in breeding places. Moreover, relative humidities were the highest for the year during these months.

This is an interesting phenomenon. At a time when humidity was lowest and temperature relatively high, a combination assumed to be most likely to have an adverse effect on adult population, there was a rapid rise, following a great increase in number of breeding places. But at a time when humidity was highest and temperature somewhat lower, a combination assumed to be likely to have a favourable effect, there was a sharp decline, although the water area available for breeding was not less. A possible explanation of this phenomenon might be as follows:—

(1) In June and July, irrigation water filled all types of breeding places, and, at that period, almost all were favourable to *A. culicifacies*. The first places to fill were irrigation canals and channels, borrowpits and ditches,



TABLE IX.

Collection data for *A. culicifacies*, at Pattukkottai, by seasons, June 1937 to January 1940, all years combined.

Months.	<i>A. culicifacies</i> collected.		Minutes collecting larvæ.		Man-hours collecting adults (routine).	<i>A. culicifacies</i> larvæ per 10 minutes collecting.		<i>A. culicifacies</i> adults per man-hour (routine).
	Larvæ.	Adults (routine).	All positive collections.	Only where <i>culicifacies</i> found.		All positive collections.	Only where <i>culicifacies</i> found.	
February to March.	3,188	731	9,191	4,185	338.5	3.5	7.6	2.2
April to May	2,822	773	9,331	2,484	414.0	3.0	11.4	1.9
June to July	12,001	4,144	16,112	9,142	585.0	7.5	13.1	7.1
August to September.	28,287	12,800	21,841	15,734	572.5	13.0	18.0	22.4
October to November.	12,715	6,367	19,567	11,617	587.5	6.5	10.9	10.8
December to January.	8,752	3,949	16,851	8,470	589.5	5.2	10.3	6.7

many tanks and most shallow irrigation wells. All these places were suitable for breeding the species. Next to be filled were enormous numbers of fallow ricefields, moderately favourable, but, by their enormous extent, responsible for a very great output. During July, many of the fields were kept fallow and flooded, and formed extensive breeding places. In August, rice was transplanted into most of the fields, and by the end of September the plants were nearly a foot high. It is known that *A. culicifacies* avoids ricefields after the plants are a foot high (Russell and Rao, 1940). Therefore, in October and November, although there was as great a water surface available for breeding, the *A. culicifacies* habitat was greatly restricted, because ricefields became unsuitable.

(2) Even in breeding places which continued to show larvæ of *A. culicifacies*, such as canals, wells, borrowpits, and field channels, there was a marked decline in the intensity of breeding from late September onwards. This is clearly shown in Chart 3 where the curve for larvæ per 10 minutes collecting shows a rapid decline. In Table X are given, as typical examples, the details of larva collections in two seepage-filled borrowpits for which records were kept in connection with some detailed investigations on the ecology of *A. culicifacies*, to be published later. They show clearly how the number of larvæ collected drops off. This phenomenon of decreased breeding in apparently suitable places is one that has been observed throughout our studies. A fourth year's observation has confirmed this, and its ecological significance will be considered



in a subsequent paper. Studies are in progress to explain the reduction in breeding after September, even in places apparently favourable to *A. culicifacies*.

TABLE X.

Total number of *A. culicifacies* larvæ collected in two special borrowpits in 1938 and 1939.

BORROWPIT No. 1.		BORROWPIT No. 2.	
Date.	Larvæ collected.	Date.	Larvæ collected.
June 29, 1938 ..	28	..	..
July 4 ..	24	..	..
July 11 ..	24	..	..
July 20 ..	39	..	..
July 25 ..	24	..	..
August 1 ..	92	August 3, 1939 ..	101
August 8 ..	67	August 10 ..	93
August 15 ..	127	August 16 ..	211
August 24 ..	157	August 22 ..	108
August 29 ..	63	August 30 ..	95
September 5 ..	128	September 7 ..	21
September 12 ..	7	September 13 ..	0
September 21 ..	19	September 19 ..	4
September 29 ..	7	September 25 ..	7
October 4 ..	8	October 5 ..	9
October 11 ..	1	October 12 ..	21
October 18 ..	12	October 18 ..	7
October 24 ..	6	October 27 ..	2
November 1 ..	2	November 2 ..	6
November 8 ..	8	November 8 ..	0
November 16 ..	0	November 17 ..	14
November 22 ..	8	November 22 ..	8
November 30 ..	0	December 1 ..	..
December 7 ..	0	December 8 ..	..
December 15 ..	4	December 15 ..	3
December 21 ..	11	December 21 ..	4
January 1, 1939 ..	2	December 29 ..	4
January 5 ..	2	January 6, 1940 ..	..
January 12 ..	1	January 13 ..	8
January 17 ..	0	January 20 ..	0
January 25 ..	49	January 27 ..	6
January 31 ..	0	February 2 ..	16

Notes.—Borrowpit No. 1 was dug on June 20, 1938.

Borrowpit No. 2 was dug on July 30, 1939.



The heavy north-east monsoon rainfall of October and November apparently has little to do with the initiation of the decline which starts well before the monsoon. Later, monsoon rains probably do affect breeding, either by interrupting irrigation or by flushing away larvæ. One might have inferred from the 1937-38 data that the August decline was due to relatively abundant rainfall in that month. But, in 1939, there were not these unusual August-September rains, yet the curve of *A. culicifacies* prevalence took its usual downward course.

Whether the declining temperature had any influence on breeding intensity is not yet certain. It is possible, however, that, from October to January when mean temperatures are below 28°C. (82.4°F.), the effect may be one of temperature. Relatively small temperature variations in the tropics may have more significance than in cooler climates.

October and November formed the peak of the malaria season, both as regards prevalence of the disease and the infection index in dissected *A. culicifacies* (Russell and Rao, 1940b).

*December-January.*—This period was the last quarter of irrigation, and was characterized by a comparatively low prevalence of *A. culicifacies*, both larvæ and adults. These were also the coolest months. The temperature, however, was never low enough to affect the ability of *A. culicifacies* to transmit malaria (Russell and Rao, 1940b), or to bring about a complete cessation of breeding, which was, however, considerably retarded by causes still to be explained. Irrigation water continued to flow into the area, although in restricted amounts.

*February-March.*—This was the first half of the non-irrigation season and was marked by low prevalence of *A. culicifacies*. The rapid drying of most of the breeding places suitable for this species may be regarded as the prime cause for the low incidence.

*April-May.*—These months were the driest ones as regards breeding places. The only habitats available for *A. culicifacies* were, wells (suitable), tanks (less suitable), and river-bed pools (suitable, but rare and hence of little consequence). The lowest densities of *A. culicifacies* were observed during these months. It is of interest to note, however, that in 1937 and 1939 there were fairly good rains in April, which caused the formation of many pools in which larvæ of *A. culicifacies* were moderately prevalent. This resulted in a slight rise in the curve in both years. In Chart 3, for instance, it can be seen that the 9.75 inches of rain which fell in April 1939, was followed by a rise in intensity of breeding, indicating that rain-water pools were favourable for the breeding of *A. culicifacies* and that the seasonal climatic conditions were also favourable. These rain-water pools, however, were transient, remaining long enough to develop one brood of *A. culicifacies* only, as reflected in the curve for adults in the same chart. Such an effect can also be seen after a less rainfall in March 1938.

This study, therefore, indicates in brief the following points regarding seasonal prevalences of *A. culicifacies* in Pattukkottai :—

(a) The period of greatest prevalence was August-September, a result apparently of the advent of irrigation water in mid-June.

(b) There was a decline in prevalence from September onwards for reasons not yet clear. It was not due to the onset of the north-east monsoon. A



similar decline was seen in prevalence of adult mosquitoes, perhaps a result of an enormous reduction in suitable breeding places by conversion of fallow into planted ricefields. The decline was also reflected in intensity of breeding, even in apparently suitable habitats. Here is an interesting ecological problem.

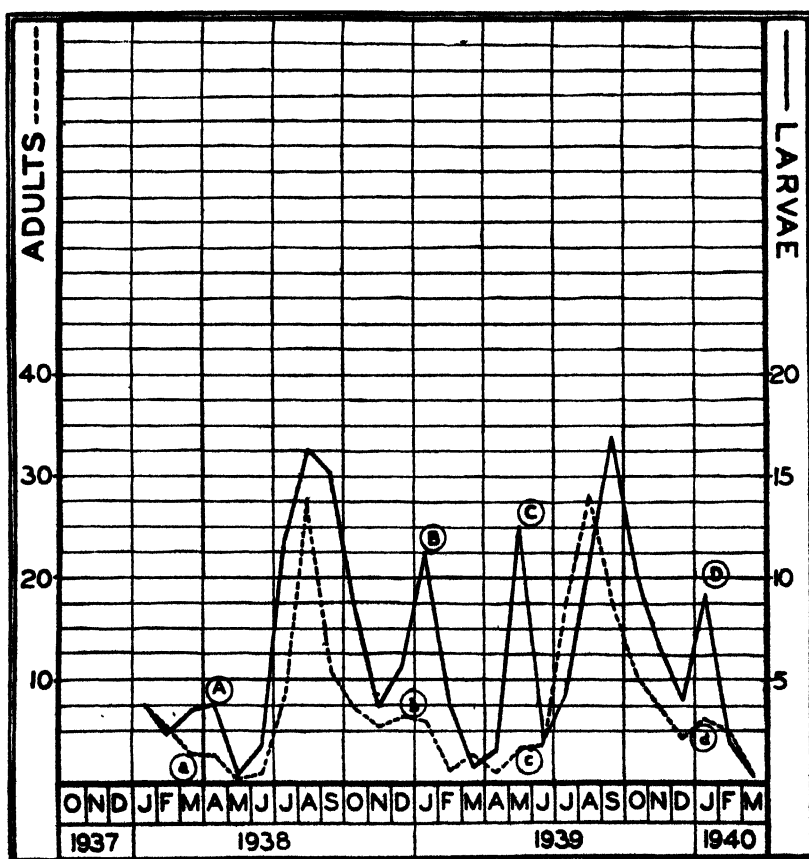
(c) The period of lowest prevalence was between February and mid-June, mainly due to a rapid drying, and relative scarcity thereafter, of breeding places, except tanks and wells. It appears, however, that had there been a sufficiency of suitable habitats, owing to rain or to a perennial irrigation programme, *A. culicifacies* would have been more abundant during these months.

#### CHART 4.

##### TUVARANGURICHCHI VILLAGE.

*A. culicifacies* larvæ and adults.

Larvæ per 10 minutes all positive collections —————  
Adults per man-hour -----



(d) Temperature and humidity did not seem to be of great consequence in this area in determining the size of the adult or larval population, except perhaps in December and January, when a lower temperature may have



retarded breeding. Any effect of low humidity in June and July was masked by the greater influence of irrigation water breeding places.

Owing to the importance of *A. culicifacies*, Table XI is presented to show collection data by months in Tuvarangurichchi, where routine studies have been continuous since October 1937. This table and the related Chart 4 are given so that a clear picture may be presented of the inter-relationship between larval and adult collections in a limited area where standardized routine collections were made. Data in other tables refer to both routine and general collections, standardized but spread over many places.

Tuvarangurichchi is a typical village where no mosquito control work has been done. There were eight adult collection stations and, associated with them, 16 larva collection stations. In Chart 4, the two curves plotted are : (1) adults of *A. culicifacies* collected per man-hour, and (2) larva of *A. culicifacies* collected per 10 minutes, in all collections positive for any species. It can be seen how closely the two curves agree regarding general trends especially peaks in the irrigation season. The points on Chart 4 marked A(a), B(b), C(c) and D(d) are interesting, in that, while there are corresponding rises in adult and larval curves, these differ in degree. It would appear that the adult curve does not show as high a rise as might have been expected, because the increase in larval density took place at a season unfavourable to adults, when breeding places were not numerous enough to mask meteorological factors.

It is of interest to note also how clearly the curves in Chart 3 agree. They represent: (1) adults of *A. culicifacies* collected per man-hour, and (2) larvæ of *A. culicifacies* collected per 10 minutes, only in places where the species was found. This chart indicates that adult curves were influenced by changes in intensity of breeding. Besides the close correspondence of general trends of the curves, there are four points marked A(a), B(b), C(c) and D(d) to which attention may be drawn. A slight rise in the intensity of breeding of larvæ in February-March 1938, was followed by a clear break in the precipitous decline of adults. These points have been marked A(a). Similar correspondence may also be noticed at the points marked B(b), C(c) and D(d).

Afridi and Puri (1940) have collected published records regarding seasonal prevalence of *A. culicifacies* in India. They classify seasonal prevalence of this species into six types. Three of these types (viz., their D\*, F and C) occur in South India. As shown in their map, the only records available indicated that Tanjore District should be included in Type F, having maximum prevalence of *A. culicifacies* in the cooler months of the year and low prevalence from June to September. The original data from Pattukkottai taluk, presented herewith, as well as intensive studies in the deltaic parts of Tanjore District, to be reported later, are not in agreement with this map.

It is of interest to note that in the Ennore-Nellore coastal tract of Madras, Russell and Jacob (1939) found the maximum prevalence of *A. culicifacies* in the winter months (Type F of Afridi and Puri, *loc. cit.*). Meteorological conditions in the winter months in Ennore are not very different from those in Pattukkottai, and it is significant that in Ennore *A. culicifacies* is increasing in November and December, while in Pattukkottai it is definitely declining. In Ennore the rise in *A. culicifacies* in the winter months may perhaps be due to

---

\* In the map in their paper the accompanying diagrammatic key is correctly labelled, but in the notes below the map, the letters D and E have been interchanged.



TABLE XI.

Routine collections of *A. culicifacies* larvæ and adults, Tuvarangurichchi village, by months, January 1938 to March 1940.

Date.	LARVÆ.							ADULTS.		
	Number of positive collections.		Total <i>culicifacies</i> collected.	Total minutes collecting.		<i>A. culicifacies</i> larvæ per 10 minutes of collection.		Total collected.	Man-hours spent collecting.	<i>A. culicifacies</i> per man-hour.
	All.	Positive for <i>culicifacies</i> .		Only where <i>culicifacies</i> found.	All collections.	All positive collections.	Only where <i>culicifacies</i> found.			
January 1938	15	9	86	135	225	3.8	6.4	73	9.5	7.7
February ..	57	29	162	383	696	2.3	4.2	69	13.0	5.3
March ..	46	24	209	304	594	3.5	6.9	32	11.5	2.8
April ..	22	10	104	125	275	3.8	8.3	29	11.5	2.5
May ..	21	3	7	30	255	0.3	2.3	3	15.0	0.2
June ..	31	8	74	110	405	1.8	6.7	9	11.5	0.8
July ..	47	34	775	480	650	11.9	16.1	96	11.5	8.3
August ..	51	42	1,102	535	670	16.4	20.6	293	10.5	27.9
September ..	16	11	310	140	205	15.1	22.1	64	6.0	10.7
October ..	46	34	452	395	545	8.3	11.4	43	6.0	7.2
November ..	29	16	120	200	335	3.6	6.0	33	6.0	5.5
December ..	28	20	171	220	305	5.6	7.8	32	5.0	6.4
January 1939	23	12	328	145	290	11.3	22.6	71	12.0	5.9
February ..	21	6	95	80	255	3.7	11.9	9	7.5	1.2
March ..	15	3	13	33	183	0.7	3.9	22	8.0	2.8
April ..	8	4	28	45	90	1.5	6.2	7	7.5	0.9
May ..	20	10	271	110	215	12.6	24.6	27	8.0	3.3
June ..	18	8	41	80	208	2.0	5.0	28	8.0	3.5
July ..	18	13	104	165	240	4.3	6.3	211	12.0	17.6
August ..	29	25	402	330	385	10.4	12.2	199	7.0	28.4
September ..	21	20	408	225	240	17.0	18.1	132	7.5	17.6
October ..	18	14	215	155	210	10.2	13.9	85	8.0	10.6
November ..	18	14	128	140	190	6.7	9.1	60	8.0	7.5
December ..	25	14	113	155	280	4.0	7.3	34	8.0	4.3
January 1940	32	20	305	200	330	9.2	15.3	73	12.0	6.1
February ..	10	5	19	50	100	1.9	3.8	10	2.0	5.0
March ..	7	2	2	20	70	0.3	1.0	6	7.5	0.8

the rise of water level in the principal breeding places—the sandy casuarina pits, due to the effect of the north-east monsoon.

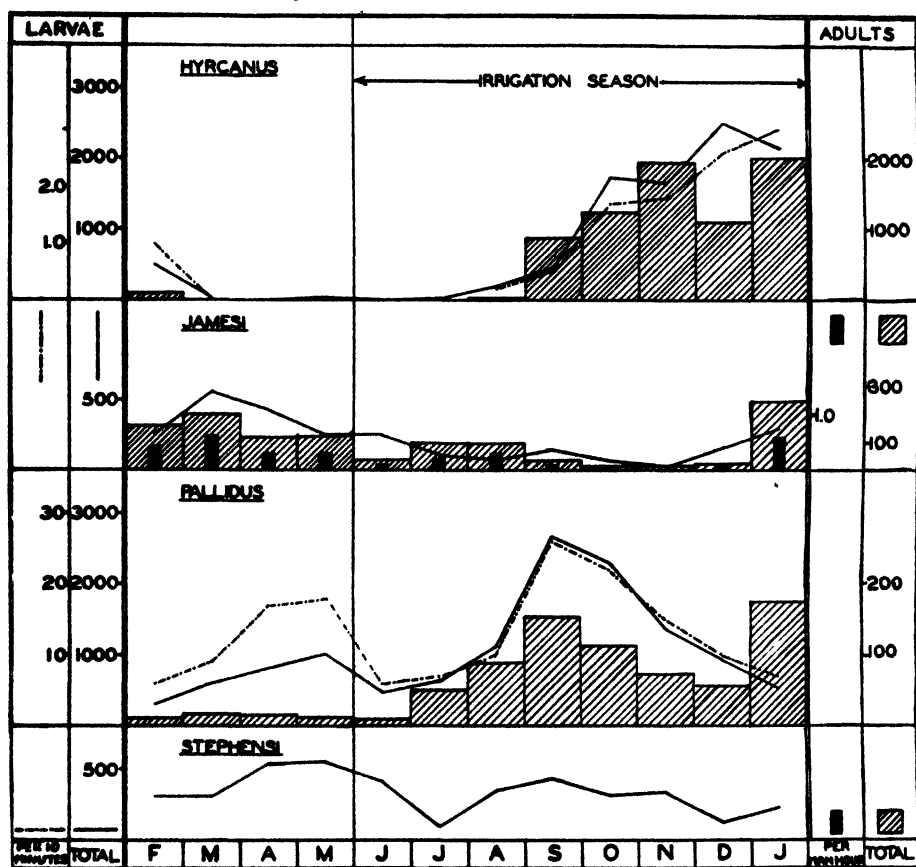
#### *A. hyrcanus* (Chart 5).

This species showed definite seasonal differences in prevalence. From March to July, both larvæ and adults were scarce and almost disappeared. From July onwards there was a steady rise, and from November to January the species became quite abundant (Chart 5). The great numbers of larvæ collected during these months came almost exclusively from ricefields. It was in the later stages of rice growth that larvæ of *A. hyrcanus* were most prevalent. When the fields were dried for harvest in January, there was a sudden drop in numbers collected.



CHART 5.

Seasonal prevalence of *A. hyrcanus*, *A. jamesi*, *A. pallidus* and *A. stephensi* as shown by collections of larvæ and adults.



Regarding adults of this species, it is not safe to rely on figures per man-hour. In the routine collections, few adults were collected in ordinary dwelling houses or cowsheds. Better collections were obtained in the portable stable trap described above. From Table VII, it will be seen that 5,339 adults of *A. hyrcanus*, all females, were collected in this trap. These collections agree with larval collections, showing a corresponding period of predominance.

The question arises as to how far the greater prevalence of *A. hyrcanus* between November and January was due to season. Undoubtedly, its great abundance during these months was mainly due to the presence of very large areas of suitable ricefields. But it may also be possible that the species could not thrive during the hotter months from March to June. However, in some ricefields in a perennial seepage area, we have collected small numbers of larvæ of this species during these months. But in the summer of 1939, observations were carried out in a number of ricefields specially planted between April and June and irrigated from a tank, and larvæ of *A. hyrcanus* were not found.



Specimens of *A. pallidus* (often associated with *A. hyrcanus* in growing rice-fields) were collected. This might indicate that the great prevalence of *A. hyrcanus* in the cooler months was due to coincidence of favourable season and favourable habitat.

*A. jamesi* (Chart 5).

This was a minor species, only 2,629 larvæ and 1,155 adults having been taken. Both larvæ and adults were found throughout the year, but both showed a slightly greater predominance in the months from January to May, a period definitely not associated with irrigation. Like *A. annularis*, its close relative and associate, the preferred habitat for breeding was tanks, but unlike this species, the period of maximum prevalence appeared to be March, fully one month earlier than *A. annularis*. The greater prevalence during these early months seemed to be definitely seasonal.

*A. pallidus* (Chart 5).

This was another species having ricefields as a preferred habitat, but it dominated the fields a month or two earlier than *A. hyrcanus*, the greatest prevalence corresponding to the middle stages of rice growth, during September-October. A study of Chart 5 shows that, as regards larvæ, in addition to a peak in September, there was another smaller rise from March to May. This secondary peak was entirely due to collections made in some hot weather rice-fields, irrigated from a tank. While these fields did not show *A. hyrcanus*, they contained *A. pallidus* larvæ in abundance. This seemed to indicate definitely that *A. pallidus* could thrive and multiply in the months from March to May, when suitable habitats were available. Its great prevalence in the irrigation season was, therefore, not so much an effect of seasonal factors, as of the abundance of preferred habitat.

Chart 5 shows a dissociation between numbers of larvæ and adults of *A. pallidus* collected during the months from March to June. This can be explained by the fact that the greater number of larvæ taken formed part of a special collection made in experimental ricefields grown in a limited area, while the figures for adults collected refer to the whole taluk.

Adults of this species were not collected in dwellings in numbers commensurate with breeding (17,705 larvæ as compared with only 774 adults). There are indications that the adults prefer to rest in outdoor places, where we have at times observed both males and females sometimes even on grass. Collections in the portable stable traps were not significantly different from those in dwellings.

*A. stephensi* (Chart 5).

This was another rare species in Pattukkottai, but it was found breeding intensely in a few wells in one town, Orathanad, isolated by several miles from other towns. Some 3,980 larvæ, but only 11 adults, of the species were collected. Although hundreds of wells in many other places were searched, the species was found only in this one town. No variations in seasonal prevalence could be detected. (It was not determined whether the specimens collected were type or var. *mysorensis*.)

*A. subpictus* (Chart 6).

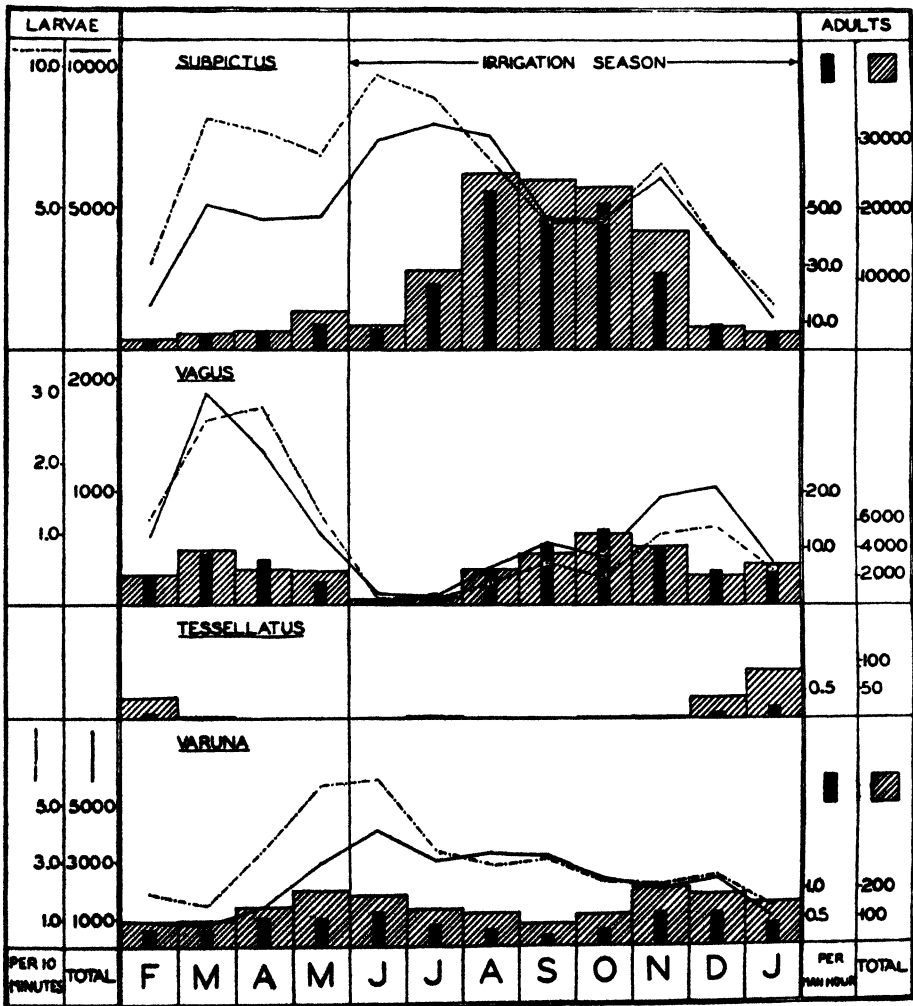
This was the most abundant anopheline, and it occurred at all seasons. Adult curves were highest between August and October. This ubiquitous



species was collected in all types of habitat. From July to September, when irrigation water flooded the country-side, it was found in enormous numbers. From November onwards there was a marked fall in the number of adults caught, for which the explanations in respect of *A. culicifacies* appear to apply.

CHART 6.

Seasonal prevalence of *A. subpictus*, *A. vagus*, *A. tessellatus* and *A. varuna* as shown by collections of larvæ and adults.



The larval curves differ somewhat. For instance, in the months from March to June, the number of larvæ collected was relatively higher than that of adults. The probable explanation is that during these months the few remaining breeding places, together with short-lived rain-water pools, were not numerous enough greatly to affect adult populations. But the intense breeding



in available places during the non-irrigation months once again indicated that the species could thrive at that season.

*A. tessellatus* (Chart 6).

This was a rare species, and adults were taken chiefly in the months from October to February. Not much was learned about its breeding habits, but the adults were found to rest commonly in outdoor wells (Rao and Russell, *loc. cit.*).

TABLE XII.

*Resting places of adult anophelines, per man-hour figures based on routine collections, in Pattukkottai town and taluk, in three types of dwellings for two years, January 1938 to December 1939.*

Species.	HUMAN DWELLINGS.		MIXED DWELLINGS.		ANIMAL DWELLINGS.		TOTAL.	
	Number collected.	Per man-hour.	Number collected.	Per man-hour.	Number collected.	Per man-hour.	Number collected.	Per man-hour.
<i>aconitus</i> ..	13	*	6	*	6	*	25	*
<i>annularis</i> ..	832	0.8	418	0.6	814	1.3	2,064	0.9
<i>barbirostris</i> ..	2	*	3	*	3	*	8	*
<i>culicifacies</i> ..	6,698	6.2	5,322	7.3	3,922	6.5	15,942	6.6
<i>hyrcanus</i> ..	12	*	12	*	99	0.2	123	0.1
<i>jamesi</i> ..	151	0.1	128	0.2	265	0.4	544	0.2
<i>pallidus</i> ..	61	0.1	45	0.1	108	0.2	214	0.1
<i>stephensi</i> ..	1	*	1	*	3	*	5	*
<i>subpictus</i> (females)	22,316	20.7	12,878	17.5	11,274	18.6	46,468	19.2
<i>tessellatus</i> ..	38	*	27	*	18	*	83	*
<i>vagus</i> (females)	6,531	6.1	4,404	6.0	3,969	6.5	14,904	6.2
<i>vagus</i> or <i>subpictus</i> (males).	22,673	21.1	11,501	15.7	5,956	9.8	40,130	16.6
<i>varuna</i> ..	314	0.3	245	0.3	214	0.4	773	0.3
Total ..	59,642	55.4	34,990	47.7	26,651	43.9	121,283	50.2

Notes.—\* Less than 0.1.

Time spent collecting .. Human dwellings = 1,076.5 hours. Mixed dwellings = 734.0 hours.  
Animal dwellings = 607.0 hours. Total = 2,417.5 hours.



*A. vagus* (Chart 6).

This species was not so abundant as its close relative, *A. subpictus*, but had similar breeding habits. *A. vagus* selected more muddy or turbid waters, preferring hoof-marks and rain-water pools. It had a more uniform seasonal distribution as regards adults; and, while it showed a rise in the irrigation season, this was not as great as in *A. subpictus*. Further, *A. vagus* became most prevalent in October, nearly two months after the peak of *A. subpictus*. Breeding became intense also during the latter half of the irrigation season. During the months of March and April, adults of *A. vagus* continued in relatively good numbers, but during June and July they were scarce. No season seemed to be unfavourable for *A. vagus*, provided suitable breeding places were available.

*A. varuna* (Chart 6).

This was a common species in wells, mainly domestic. Adults were less abundantly captured. There were no clear indications of adult seasonal prevalences, but the larvæ showed a distinctly greater prevalence during May and June.

TABLE XIII.

*Sex ratio of adult Anopheles, by species, March 1937 to February 1940, routine collections only.*

Species.	Males.	Females.	Total	Ratio of males to females.
<i>aconitus</i> ..	3	24	27	1 to 8.0
<i>annularis</i> ..	622	2,213	2,835	1 to 3.6
<i>barbirostris</i> ..	2	12	14	1 to 6.0
<i>culicifacies</i> ..	9,566	20,390	29,956	1 to 2.1
<i>hyrcanus</i> ..	6	161	167	1 to 26.8
<i>jamesi</i> ..	163	620	783	1 to 3.2
<i>pallidus</i> ..	54	263	317	1 to 4.9
<i>stephensi</i> ..	3	6	9	1 to 2.0
<i>subpictus</i> ..	..	68,780	68,780	*
<i>tessellatus</i> ..	24	115	139	1 to 4.8
<i>vagus</i> ..	..	22,299	22,299	*
<i>varuna</i> ..	285	1,027	1,312	1 to 3.6
<i>subpictus</i> or <i>vagus</i> (males).	56,196	..	56,196	*
TOTAL ..	66,924	115,910	182,834	..

\* Ratio of males to females of *subpictus* and *vagus* combined was 1 to 1.6.



## SUBSOIL WATER LEVELS.

In Table XIV are shown monthly subsoil water levels, for 1938 and 1939. We have not found any direct correlation between these levels and the incidence of anopheline breeding, but the high levels undoubtedly help to keep such breeding places as borrowpits full of water.

## ADULT RESTING PLACES.

In Table XII are given data for anophelines collected in the three major types of dwellings, namely, human, animal, and mixed. These figures, given as average numbers collected per man-hour, over a full two-year period (1938 and 1939), refer exclusively to routine catching stations. Dwellings were in all cases mud and thatched huts, typical of the area. Mixed dwellings were those in which cattle were sheltered at night under the same roof as humans, and in which there was free access of mosquitoes to migrate from one section to the other.

TABLE XIV.

*Subsoil water level in 25 wells in Pattukkottai town.*

Month.	Average depth in feet below ground level.	
	1938.	1939.
January ..	4.70	3.86
February ..	5.23	5.00
March ..	5.58	6.30
April ..	7.45	5.98
May ..	9.68	5.32
June ..	10.70	6.63
July ..	8.50	6.36
August ..	6.18	5.13
September..	4.88	5.58
October ..	4.94	3.54
November..	3.10	1.68
December ..	3.52	2.43

*Note.*—Measurements were taken once a week.

The figures indicate that, so far as daytime resting habits were concerned, *A. culicifacies* did not show any significant preferences as between the three types of dwellings. *A. annularis*, *A. hyrcanus*, *A. jamesi* and *A. pallidus* seemed to have a slight preference for animal dwellings (cowsheds). *A. subpictus* females were a little more common in human dwellings. So, too, males of



PLATE XXX.



Fig. 1 Typical adult *Anopheles* collecting station, Pattukkottai.



Fig. 2 Typical adult *Anopheles* collecting station, Pattukkottai



PLATE XXXI.



Fig 3 Portable stable trap for *Anopheles* adults (Magoon, 1935, type)



*A. subpictus* or *A. vagus* (indistinguishable morphologically) were more abundant in human dwellings than in cowsheds. The figures for *A. varuna* show a uniformity in the three types of dwellings.

The small number of *A. hyrcanus* collected in dwellings and its great abundance in traps, facts which have already been mentioned, seemed to be due to a preference of the females of this species to rest in outdoor places.

As noted above, *A. pallidus* was seen in outdoor resting places, and this may explain the low numbers caught in dwellings, compared with the enormous amount of breeding in ricefields during the irrigation season. *A. tessellatus* rested outdoors, and was commonly found in open wells (Rao and Russell, *loc. cit.*).

### RATIO OF SEXES.

In Table XIII are given totals of adults collected, in routine stations only, during the three years, showing the ratio of sexes. In every species, more females than males were caught. In the case of *A. hyrcanus*, the ratio was highest (26.8 to 1). The ratio for *A. culicifacies* was 2.1 to 1 and for *subpictus-vagus* 1.6 to 1.

### CONCLUSIONS.

Briefly, the periods of prevalence of Anopheles species in the newly irrigated tract of Pattukkottai taluk, Tanjore District, south-eastern Madras, were as follows :—

Species.		Greatest prevalence.	Comments.
<i>A. aconitus</i>	.. ..	February to March	Absent in months May to December.
<i>A. annularis</i>	.. ..	March to July, peak in April and May	But occurs throughout the year.
<i>A. barbirostris</i>	.. ..	November to March, peak in January	Very rare in other months.
<i>A. culicifacies</i>	.. ..	July to October, peak in August	Occurs throughout the year and can thrive in February to June, provided there are suitable breeding places.
<i>A. hyrcanus</i> var. <i>nigerrimus</i>		November to January, peak in December to January	Very scarce in months February to June.
<i>A. jamei</i>	.. ..	January to May	Occurs throughout the year.
<i>A. pallidus</i>	.. ..	September to October, peak in September	Scarce in months February to June, but can thrive if suitable habitat available.
<i>A. stephensi</i> (type or var. <i>mysorensis</i> ?)		No seasonal prevalences noticed	
<i>A. subpictus</i>	.. ..	July to November, peak in August	Occurs throughout the year, and can thrive from February to June if there are breeding places.
<i>A. tessellatus</i>	.. ..	December to February, peak in December	Scarce in other months.
<i>A. vagus</i>	.. ..	August to November, peak in October	Occurs throughout the year, and can thrive from February to June, if breeding places available.
<i>A. varuna</i>	.. ..	No seasonal variations noted	Occurs throughout the year.



## SUMMARY.

Data are presented from intensive collections of *Anopheles* larvæ and adults during a three-year period from March 1937 to February 1940, in the newly irrigated area of Pattukkottai taluk, Tanjore District, south-eastern Madras. Seasonal prevalences of the following twelve species are discussed: *A. aconitus*, *A. annularis*, *A. barbirostris*, *A. culicifacies*, *A. hyrcanus* var. *nigerrimus*, *A. jamesi*, *A. pallidus*, *A. subpictus*, *A. stephensi*, *A. tessellatus*, *A. vagus* and *A. varuna*. Meteorological and seasonal conditions are described and their relation to *Anopheles* prevalence, with special reference to *A. culicifacies*, is discussed.

## REFERENCES.

- AFRIDI, M. K., and PURI, I. M. Studies on the behaviour of adult *Anopheles culicifacies*. Part I. Review of literature. *J. Mal. Inst. Ind.*, **3**, 1, pp. 1-22.
- HICKS, E. P., and MAJID, S. A. A study of epidemiology of malaria in a Punjab District. *Rec. Mal. Surv. Ind.*, **7**, 1, pp. 1-46.
- MAGOON, E. H. (1935) .. A portable stable trap for capturing mosquitoes. *Bull. Ent. Res.*, **26**, 3, pp. 363-370.
- RAO, T. R., and RUSSELL, P. F. (1940). A note on wells as daytime resting places of *A. tessellatus*. *Ind. Med. Gaz.*, **75**, 11, pp. 679-680.
- RUSSELL, P. F., and JACOB, V. P. Epidemiology of malaria in the Ennore-Nellore coastal area, Madras Presidency, India. *J. Mal. Inst. Ind.*, **2**, 2, pp. 131-152.
- RUSSELL, P. F., MENON, M. K., and RAO, T. R. (1938). Epidemiology of malaria in Pattukkottai taluk, Tanjore District, Madras Presidency, India. *Ibid.*, **1**, 1, pp. 285-326.
- RUSSELL, P. F., and RAO, H. R. The *Anopheles* of ricefields in south-eastern Madras. *Ibid.*, **3**, 4, pp. 427-446.
- RUSSELL, P. F., and RAO, T. R. (1940a). On habitat and association of species of anopheline larvæ in south-eastern Madras. *Ibid.*, **3**, 1, pp. 153-178.
- Idem* (1940b). Natural malaria infections in some South Indian anophelines. *Ibid.*, **3**, 4, pp. 543-562.
- RUSSELL, P. F., RAO, T. R., and JACOB, V. P. (1938). *Anopheles subpictus* Grassi, 1899, and *Anopheles vagus* Dönitz, 1902, found naturally infected with malaria Plasmodia in south-eastern India. *Ibid.*, **2**, 1, pp. 95-99.



## MALARIA-CARRYING ANOPHELINES IN ASSAM, WITH SPECIAL REFERENCE TO THE RESULTS OF TWELVE MONTHS' DISSECTIONS.

BY

D. K. VISWANATHAN, L.M.S., B.S.Sc , M.P.H. (Johns Hopkins),  
*Research Officer,*  
SRIBAS DAS,  
*Malariologist,*

AND

A. V. OOMMEN,  
*Entomological Assistant.*  
(*Assam Medical Research Society, Shillong.*)

[October 3, 1941.]

### INTRODUCTION.

IN the résumé of the activities of the Assam Medical Research Society during the period 1931 to 1941, the results of dissections of all anophelines were summarized by the senior author (Anderson and Viswanathan, 1941). The total dissections numbered 70,049 out of which 774 specimens showed infections, 409 gland and 425 gut infections. Six species were found infected, namely *A. aconitus* (1 gut out of 1,145), *A. annularis* (6 glands and 3 guts in 9 specimens out of 16,760), *A. culicifacies* (5 glands and 2 guts in 6 specimens out of 1,232), *A. maculatus* and its variety *willmori* (5 glands and 24 guts in 29 specimens out of 8,483), *A. minimus* (391 glands and 393 guts in 725 specimens out of 14,092), and *A. philippinensis* (2 glands and 2 guts in 4 specimens out of 4,239). Reference was also made to the finding of infections in *A. leucosphyrus* in Digboi by the Medical Officer of the Assam Oil Company which has since been recorded by Clark and Choudhury (1941).

The present paper describes the results of dissections of 18,599 anophelines received from 25 centres in the province and dissected in the Society's laboratory at Shillong during the period July 1940 to June 1941.

### RESULTS OF DISSECTIONS (ALL CENTRES).

Table I shows the results of dissections, by months, of the specimens received from all centres.







<i>A. barbirostris</i> ..	1	24	5	2	3	4	12	6	21	3	4	4	89
<i>A. culicifacies</i> ..	3	1	1	..	8	1	3	1	7	1	6	7	39
<i>A. fluviatilis</i> ..	..	..	..	..	..	..	15	..	1	..	..	..	16
<i>A. gigas</i> ..	2	6	1	1	..	1	1	..	1	..	3	2	18
<i>A. hyrcanus</i> ..	30	19	26	8	28	2	19	8	5	..	8	8	161
<i>A. jeyporiensis</i> ..	..	..	..	..	..	..	..	1	1	..	..	..	2
<i>A. kochi</i> ..	..	3	..	1	..	..	..	..	..	..	..	..	4
<i>A. leucosphyrus</i> ..	2	..	..	..	..	..	..	..	..	..	..	..	2
<i>A. pallidus</i> ..	1	..	..	..	..	..	1	4	..	..	3	3	12
<i>A. philippinensis</i> ..	4	15	15	1	3	..	..	1	..	..	..	..	39
<i>A. splendidus</i> ..	..	..	..	..	..	..	..	1	3	..	4	..	8
<i>A. subpictus</i> ..	2	98	1	..	..	1	2	2	3	11	114	119	353
<i>A. umbrinus</i> ..	..	..	..	..	..	..	..	..	..	..	..	2	2
<i>A. vagus</i> ..	257	1,118	612	53	71	20	51	19	1	12	530	652	3,396
<i>A. varuna</i> ..	..	..	19	5	2	..	2	2	..	..	..	..	30
TOTAL ..	1,851	2,915	2,477	1,848	2,084	1,096	1,240	1,021	655	387	1,241	1,784	18,599



During this period four species have been found infected, namely *A. aconitus*, *A. annularis*, *A. maculatus* and *A. minimus*. No infections were met with in two other species found infected in the past, namely *A. culicifacies* and *A. philippinensis*. The rôle of these six species as possible vectors in this province will now be discussed.

*A. aconitus*.—Out of 1,145 specimens dissected during the period 1931 to 1940, only one gut infection was encountered in September 1940 in an area (Lakhipur) where *A. annularis*, as shown later, is deemed to be the main vector. *A. aconitus* is not, therefore, considered to be a vector of any importance in Assam.

*A. culicifacies*.—This has been found infected only in Lumding (Assam-Bengal Railway), a large railway colony. Six infections (4 in glands, 1 in gut and 1 in both) were encountered out of 1,232 specimens. *A. minimus* is a more important vector in Lumding and quite possibly the infections in *A. culicifacies* may be looked upon as 'overflow' infections.

Afridi and Puri (1940) have in their studies on the behaviour of adult *A. culicifacies* summarized an extensive literature relating to their seasonal prevalence, resting places, feeding habits, hibernation and aestivation, longevity and flight dispersion and infiltration. Referring to Assam, they state that *A. culicifacies* exists only in small numbers and that it does not play an important rôle in the transmission of malaria. Our experience is in full accord with the above conclusion. The fact, however, that it was found infected in Lumding shows that, if the assumption of Senior White (1940), that *A. culicifacies* is a mixture of two as yet morphologically indistinguishable races only one of which is a vector, is correct, the vector race is not absent in Lumding but its rôle in transmission is small due to the small numbers present.

Senior White (*loc. cit.*) has reviewed the results of dissections of this species in the Indian subcontinent, and concludes that it is an active vector in the Trans-Indus and Ceylon areas, and that between the Indus and the upper course of the Ganges, and in the Burmese area and in Peninsular India south of the Godavari the sporozoite rate is in the first point of decimals, but it is a primary vector on account of its numerical prevalence. Assam would come under this region, but Senior White has qualified his conclusion earlier that in this region it is a vector of importance wherever it is found in large numbers. Lumding is the only place where it has been found infected. The species is never prevalent in large numbers in any part of the province. Hence we do not consider it as a vector of importance in Assam.

*A. philippinensis*.—This species was found infected in 1931 and 1933 and never since. From 1934 to 1940, 1,254 specimens were dissected with no infections. There is, therefore, no evidence that this species is a vector of any importance in Assam.

*A. annularis*.—Till the end of June 1941 altogether 20,043 dissections were made among which 11 infections (7 glands and 4 guts) have been encountered. The total infection index is only 0.05 per cent, but the infections were met with for the first time only in 1940, and it will be seen from Table I that the total infection index in 1940–41 is 0.15 per cent (11 out of 7,481). Besides, it is the most numerically prevalent species in the area where it is found infected, and no other species has been found infected there. For these reasons, it is considered that it is a vector of definite importance in that area (Gauripur and Lakhipur in Goalpara District). Table I shows the seasonal infection in this



species, and although, in view of the small number of infections met with in different months, it is not safe to assess its seasonal infection, the finding of sporozoites in February shows that active transmission may occur during that month when both the average and minimum daily temperatures are below 60°F. Presumably the temperature of the microclimate is much higher.

*A. maculatus* (type and var. *willmori*).—This species has been found infected in 1932, 1933 and 1934 and again in 1940–41. But these were the only periods when they were subjected to detailed study in connection with the malaria survey of Shillong and its environs. In the ten-year period 1931 to 1941, 25 gut and 5 gland infections have so far been encountered out of a total of 8,952 dissections. The total infection index is, therefore, 0.34 per cent. In 1940–41, 13 gut and one gland infections were met with in 1,573 dissections with a total infection index of 0.96 per cent.

In the year 1932, *A. minimus* was the main vector in Shillong, and the finding of infection in *A. maculatus* as well may relegate it to the rôle of a secondary carrier of little importance. In 1940–41, however, it is the only vector which has been found and *A. minimus* was present in small numbers, only 4 larvæ and 9 adults being included in our collections. The infant malaria parasite rates were nil (out of 85) in April 1940, nil in August 1940 (out of 89), 2.6 per cent in November 1940 (out of 38) and 1.5 per cent in February 1941 (out of 133). The infants found infected in November 1940 and February 1941 were born in Shillong and gave no history of having left the station at any time. Hence malaria transmission did occur in Shillong in 1940 and human Plasmodia were found only in *A. maculatus*. This species is, therefore, reckoned as a definite vector in Shillong. It is, however, capable of causing only low grade endemicity, since the spleen rate in children from 2 to 10 years old was only 1.5 per cent (16 out of 1,050). This is presumably due to its great preference normally for bovine blood. Out of 158 blood specimens from the gut of this species, 110 were positive with bovine antisera and none with human. The number of tests is, however, small and a small proportion at least may be deemed to feed on human beings in view of the finding of human Plasmodia both in gut and glands.

*A. minimus*.—During the ten-year period ending with June 1941, 14,906 *A. minimus* were dissected and 754 were found infected with a total infection index of 5.1 per cent. Amongst the infections recorded, 404 were in glands with a sporozoite index of 2.7 per cent and 409 in guts with an oöcyst index of 2.7 per cent.

During 1940–41, in 5,120 dissections there were 72 gland and 83 gut infections and 154 total infections. The oöcyst index was, therefore, 1.6 per cent, the sporozoite index 1.4 per cent and total infection index 3.0 per cent.

### SEASONAL INFECTIONS.

Table II shows the number of *A. minimus* dissected each month, the number found infected (gland, gut and total), the sporozoite, oöcyst and total infection indices, the expected number of infections each month on the assumption that there was no seasonal variation and the square of the difference between the expected and the actual infections each month divided by the expected infections.

The sum of the total deviations from expected occurrences of infection each month is 64.6 and the probability of this being exceeded is less than 0.01 for



TABLE II.  
Seasonal infection in *A. minimus*.

Month.	Number dissected.	Guts positive.	Glands positive.	Oöcyst index, per cent.	Sporozoite index, per cent.	Total positive.	Total infection index, per cent.	Expected total positive.	$\frac{(T-O)}{T}$
January ..	153	0	2	0.0	1.3	2	1.3	4.6	1.5
February ..	132	0	0	0.0	0.0	0	0.0	4.0	4.0
March ..	43	3	2	7.0	4.7	5	11.6	1.3	10.5
April ..	69	2	3	2.9	4.3	5	7.2	2.1	4.0
May ..	121	1	3	0.8	2.5	4	3.3	3.6	0.4
June ..	294	10	3	3.4	1.0	13	4.4	8.8	2.0
July ..	952	3	9	0.3	0.9	12	1.3	28.6	9.6
August ..	641	15	14	2.3	2.2	28	4.4	19.2	4.0
September ..	873	10	8	1.1	0.9	18	2.1	26.2	2.6
October ..	873	34	15	3.9	1.7	49	5.6	26.2	19.8
November ..	672	5	11	0.7	1.6	16	2.4	20.2	0.9
December ..	297	0	2	0.0	0.7	2	0.7	8.9	5.3
TOTAL ..	5,120	83	72	..	..	154	3.0	..	..

T = Theoretical infections.

O = Observed infections.

11 degrees of freedom (Fisher, 1930). Hence the variations in seasonal infections are significant. Such variations, however, may be due to possible differences in the seasonal infection in the different geographical areas from where specimens have been received, different proportions of specimens received from place to place and month to month and to the grouping of such different experiences. The monthly figures dissected from each individual centre are too small to analyse the variance in seasonal infection for each place with precision. During the period 1940-41 one centre, Nongpoh, was kept under very close observation. The figures for this centre are shown in Table III.

In this centre, transmission occurs from April to November. Although the highest index was recorded in July (13.0 per cent), October may be reckoned as the worst month by reason of the largest numerical prevalence of the species with a fairly high infection index. The absence of infection met with from December to March in the above series is not necessarily due to that period being unsuitable for transmission, since only small numbers were dissected. In December when the highest number was dissected, during the period December to March,



TABLE III.

*Seasonal infection in A. minimus in Nongpoh, 1940-41.*

	1940.						1941.						Total.
	July.	August.	September.	October.	November.	December.	January.	February.	March.	April.	May.	June.	
Number dissected	23	179	370	694	129	45	18	25	4	21	38	76	1,622
Number positive	3	19	11	39	3	..	..	..	..	2	2	4	83
Infection index, per cent.	13.0	10.6	3.0	5.6	2.3	..	..	..	..	9.5	5.3	5.3	5.1
Glands ..	1	7	1	9	1	..	..	..	..	2	2	2	25
Guts ..	2	13	10	30	2	..	..	..	..	0	0	2	59

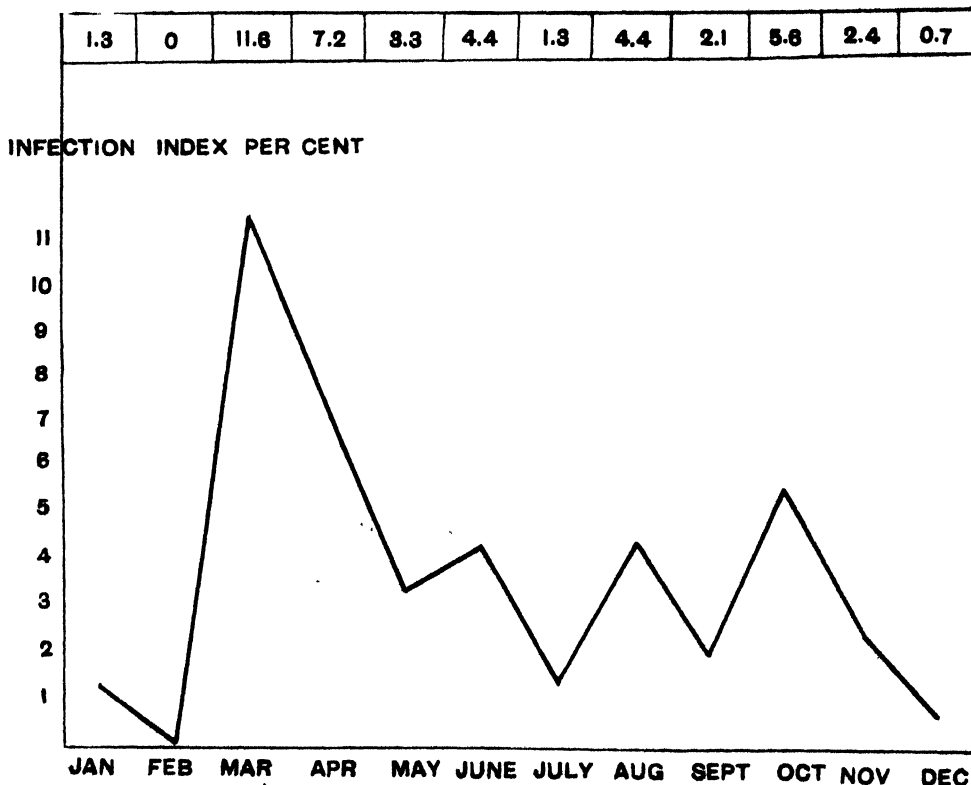
the probability of encountering at least one infection was 0.91, assuming that the true infection index for that month was 5.1 per cent, which is the index for the whole year. Hence no infection may be met with in 9 per cent of the trials, and the absence of infection in December may be due to chance. In the other months, since even smaller numbers have been dissected, the absence of infections may be due to chance to a greater degree. The number of specimens dissected each month may be reckoned as expressing the seasonal adult prevalence, except the figure for July, since some days were lost in determining the catching stations and arranging facilities for daily transport of Barraud boxes. In this centre, there is no perennial stream close by, which accounts for the low adult prevalence in the cold months. So far as this centre is concerned, our conclusions are that (1) active transmission occurs from April to November with the peak in October, and (2) the absence of transmission in the cold weather from December to March is mainly due to the paucity of adult prevalence on account of lack of perennial breeding grounds. The available data are not adequate to show conclusively whether such absence of transmission in the cold weather is also due to the unsuitability of that period.

From the total figures set out in Table II it will be observed that infections have been met with in every month excepting February. On the general experience of a 3 per cent infection index, the probability of getting at least one infection among 132 specimens dissected in February is 0.97. Hence no infection may be met with only in 3 per cent of trials, and the absence of infection in February may, therefore, be deemed to be significant that conditions during this month are not suitable for transmission. In March, the infection index suddenly rises to 11.6 per cent. It is in this month that the geographical factor plays an important part. All the five infections in March were encountered only in one centre, viz., Tangla (Eastern Bengal Railway) in Darrang District on the north bank of the Brahmaputra. Three out of the five infections in April were also from this centre. In the hot plains, where the cold weather is of shorter duration, transmission definitely commences in the month of March.



In all the centres transmission occurs from March or April till the end of the year with a peak in October owing to the combination of very high adult prevalence and a fairly high infection index. The infection index curve (Graph 1) shows a quadri-modal amplitude, the first in March, the second in June, the third in August and the fourth in October, while the curve of adult prevalence (Graph 2) is bimodal with an almost flat peak from July to October except for a small notch in August. The occurrence of successive waves of transmission with increasing amplitude has been described by one of us in epidemic malarial prevalence (Viswanathan, 1936), and the periodicity of these successive waves

GRAPH 1.

Seasonal infection in *A. minimus*, Assam, 1940-41.

was attributed to units of interval of communal transmission of the disease. The tendency for such successive waves of infection is now shown to be manifest also under conditions of static endemic malarial prevalence, though it is naturally not attended with the same degree of increasing amplitude.

## SUMMARY.

(1) The results of dissection of 18,599 anophelines from different centres in the province of Assam during the period July 1940 to June 1941 are summarized,

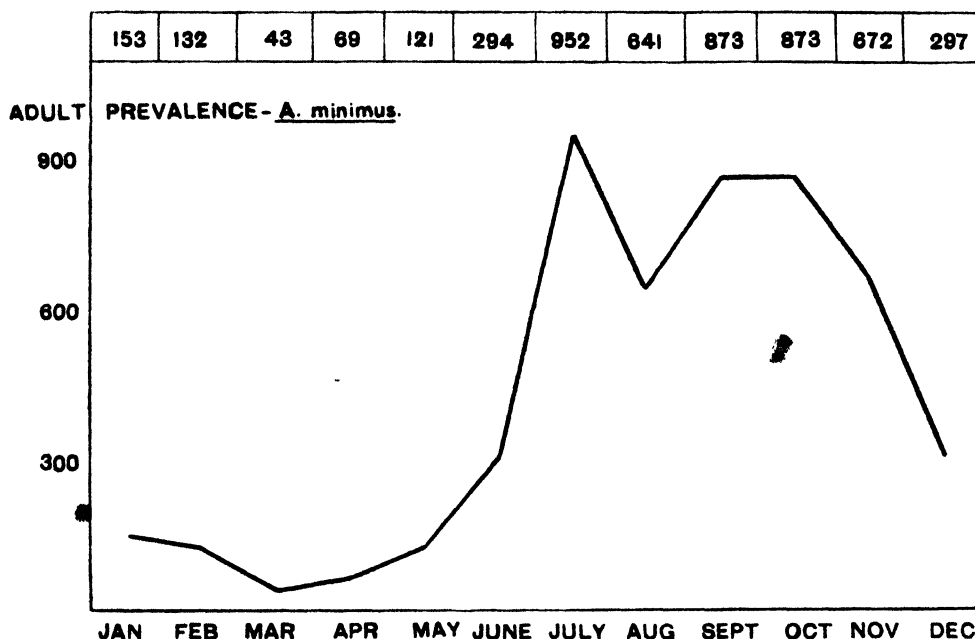


the rôle of the different vector species is described and the seasonal infection in *A. minimus* is discussed.

(2) *A. minimus* is the most important vector in Assam, with a sporozoite index of 1.4 per cent, oöcyst index of 1.6 per cent and total infection index of

GRAPH 2.

Seasonal adult prevalence in *A. minimus*, Assam, 1940-41.



3.0 per cent. In certain limited areas, however, other vectors are responsible for the low grade endemicity met with. Thus, *A. maculatus* is reckoned as a vector causing a small degree of malaria in Shillong. Despite its numerically large prevalence, its feeble efficiency as a vector is due to a large proportion of the species preferring bovine blood. *A. annularis* is deemed to be a vector in certain parts of Goalpara District (Gauripur and Lakhipur). *A. culicifacies*, though found infected in the past in small numbers, is not reckoned as a vector of importance, due to its very low numerical prevalence. Though *A. philippinensis* has previously been recorded as infected, no infections have been encountered in the last few years.

(3) In one centre, Nongpoh, *A. minimus* was found infected from April to November, with the largest number of infections in October. The absence of infection in December to March may be due to the known paucity of its prevalence, or to the season being unfavourable for transmission, but there is no conclusive evidence for the latter view. In the whole series, the infection curve has a quadri-modal amplitude in March, June, August and October with the largest number of infections in October. The absence of infection in February is shown to be significant and presumably due to the extreme cold



weather conditions then prevalent. March figures are considered erratic due to variations in different geographical areas.

#### ACKNOWLEDGMENT.

The authors thank Lieut.-Colonel L. A. P. Anderson, I.M.S. (Retd.), Honorary Secretary of the Assam Medical Research Society for permission to publish this paper.

#### REFERENCES.

- AFRIDI, M. K., and PURI, I. M. *J. Mal. Inst. Ind.*, **3**, 1, pp. 1-19. (1940).
- ANDERSON, L. A. P., and VISWANATHAN, D. K. (1941). *The Assam Medical Research Society, Shillong. A résumé of its activities during 1931-41.* Thacker, Spink and Co (1933), Ltd., Calcutta.
- CLARK, R. H. P., and CHOUDHURY, M. A. (1941). *J. Mal. Inst. Ind.*, **4**, 1, pp. 103-107.
- FISHER, R. A. (1930) .. .. *Statistical methods for research workers.* Oliver and Boyd, London.
- SENIOR WHITE, R. (1940) .. .. *J. Mal. Inst. Ind.*, **3**, 2 and 3, pp. 363-382.
- VISWANATHAN, D. K. (1936) .. .. *Rec. Mal. Surv. Ind.*, **6**, 2, pp. 239-271.



## **ABSTRACT.**

### **MALARIA IN THE JHARIA MINING SETTLEMENT, BIHAR\*.**

BY

G. R. RAO, L.M.P., D.T.M.

(*Antimalaria Officer, Jharia Mines Board of Health, Dhanbad.*)

[June 6, 1941.]

THE observations on which this paper is based were carried out during the period April 1938 to September 1940. The settlement covers an area of 787 square miles, two-thirds of which is classed as rural, whilst the remaining one-third represents the actual coal-mine section. It occupies the Dhanbad Subdivision of Manbhum, one of the five districts of the Chota Nagpur Division of Bihar Province. The land is described as undulating and porous with a gentle slope from north-west to south-east, and is traversed by numerous nalas called *jhores*, most of which are completely dry during a great part of the year. These discharge into the Damodar River, and besides affording natural drainage for stormwater, serve as the main offtake channels for the underground water pumped up from the coal-mines. The average annual rainfall is about 50 inches, most of which falls during the period of the south-west monsoon (May to October). The population of the whole settlement is approximately 550,000, of which the coalfields section contributes 160,000.

There is a well-marked malaria season, commencing in May, the curve of incidence usually exhibiting a peak in August or September (Table I).

There has been a progressive increase in the number of malaria cases recorded among the employees of the collieries (Table II) in recent years.

No information is given regarding the number of persons employed each year, but one of the reasons put forward for the enhanced malaria incidence is a great influx of new labourers following an increase in the local mining activities. Table II, therefore, gives a less accurate picture of the increase in malaria incidence than if the figures were expressed as rates per thousand. A second reason given for the increase in malaria is the practice of de-pillaring the mines which has led to the formation of a large number of depressions throughout

---

\* A copy of the original MS. has been placed in the library of the Malaria Institute of India, Kasauli, and is available on loan to workers who wish to consult it. (*Editor.*)



the coalfields area in which water collects and provides breeding places for mosquitoes.

TABLE I.

*Monthly malarial incidence in the coalfields area, as reported to the Jharia Mines Board of Health, by all the collieries within the Jharia Mining Settlement.*

	1935.	1936.	1937.	1938.	1939.
January ..	471	926	950	1,163	1,444
February ..	456	897	674	1,268	1,428
March ..	497	884	1,070	1,158	1,905
April ..	509	839	1,487	1,205	2,150
May ..	509	844	1,791	1,569	1,910
June ..	529	1,795	2,049	3,622	1,695
July ..	1,159	6,104	3,589	7,548	3,297
August ..	2,256	8,163	5,949	7,306	5,920
September ..	3,187	5,927	7,658	6,595	6,617
October ..	2,608	3,643	4,781	4,928	3,251
November ..	1,922	2,053	2,579	2,949	1,970
December ..	1,146	1,605	1,414	1,679	1,272

TABLE II.

Year.	Malaria cases.
1933	8,067
1934	9,096
1935	15,249
1936	33,680
1937	33,991
1938	40,990
1939	32,859

Spleen rates in different collieries showed wide variations from zero to 80 per cent, the highest rates being confined to a central belt traversing the coalfields section from east to west.



Figures relating to collections and dissections of adult mosquitoes are given in Table III.

TABLE III.

Species.	Number collected.	Number dissected.	NUMBER INFECTED.	
			Gut.	Gland.
<i>A. fluviatilis</i> ..	994	813	34 (4.2%)	16 (2.0%)
<i>A. stephensi</i> ..	536	423	5 (1.2%)	7 (1.7%)
<i>A. annularis</i> ..	884	798	5 (0.6%)	2 (0.25%)
<i>A. culicifacies</i> ..	191	33	0	0
<i>A. subpictus</i> ..	2,130	1,625	0	0
<i>A. vagus</i> ..	2,235	1,843	0	0
<i>A. splendidus</i> ..	18	6	0	0
<i>A. barbirostris</i> ..	8	8	0	0
<i>A. hyrcanus</i> ..	20	20	0	0
<i>A. jeyporiensis</i> ..	3	3	0	0
<i>A. pallidus</i> ..	1	1	0	0
<i>A. umbrosus</i> * ..	1	1	0	0

Antimalaria measures included naturalistic methods (unspecified), and the application of oil and paris green to breeding places. The spray-killing of adult mosquitoes with pyrethrum insecticide was also encouraged. In collieries where these measures have been systematically applied, a marked reduction in malaria incidence has been effected.

G. C.

\* The occurrence of this species in the Jharia Coalfields is exceedingly unlikely, and this record cannot be accepted without further confirmation.







***ERRATA.***

**Vol. III, 2 and 3, September 1940.**

**Page 293, 7th line from bottom, read *vagus* for *vadus*.**

**Page 327, 10th line from bottom, read 32.2 for 22.2.**







ACTIVE IMMUNIZATION OF FOWLS AGAINST SPOROZOITES  
BUT NOT TROPHOZOITES OF *PLASMODIUM*  
*GALLINACEUM* BY INJECTIONS OF  
HOMOLOGOUS SPOROZOITES\*.

BY

PAUL F. RUSSELL

(*Field Director, International Health Division of the Rockefeller Foundation*),

LIEUT.-COLONEL H. W. MULLIGAN, I.M.S.,

AND

BADRI NATH MOHAN

(*Senior Technical Assistant, Malaria Investigations*).

[December 6, 1941.]

INTRODUCTION.

THIS paper reports a further series of observations on the partial protection of fowls against mosquito-borne infection with *Plasmodium gallinaceum*, after repeated injections of inactivated sporozoites had considerably increased the agglutination titre of the fowl's sera to homologous sporozoites. It also reports contrasting observations which showed an absence of even partial protection against infection by blood inoculation of homologous trophozoites, in fowls whose agglutination titre had been increased in the same way by injections of inactivated sporozoites. The description of another method of inactivating sporozoites while retaining their specific agglutinogenic properties is also included.

Reference may be made to previous papers (Mulligan, Russell and Mohan, 1940, 1941; Russell, Mulligan and Mohan, 1941) for a discussion of the specific character of sporozoite agglutination, the agglutinogenic properties of inactivated sporozoites of *P. gallinaceum*, the first series of observations demonstrating active immunization of fowls by injections of killed homologous sporozoites, and for descriptions of technique. The last paper of the series (Mulligan, Russell

---

\* These studies were made under the auspices, and with the support, of the International Health Division of the Rockefeller Foundation, co-operating with the Pasteur Institute of Southern India. The authors are indebted to P. Balarama Menon for technical assistance.



and Mohan, 1941) also included a discussion of the dual cellular and humoral mechanism of defence against malaria, and of the observations of other investigators with regard to immunity phenomena in avian malaria.

### MATERIALS AND METHODS.

With two exceptions, materials and methods were the same as those employed previously and described in the three papers cited above. The first change in technique was in the method of inactivating sporozoites used in fowls 176, 177 and 185 (Table I). Instead of exposing dissected salivary glands to ultra-violet radiation, as was done in all previous cases, sporozoites were inactivated by grinding and drying the thoraces of infected mosquitoes, without dissecting out the glands. Thoraces, cleaned of wings and legs, were ground in a small mortar and when the pulverized mass was entirely dry, normal saline was added and the suspension was injected intravenously. Seven injections, totalizing from 180 to 220 thoraces, were given, resulting in considerable increases in agglutination titres (Table I). This method will be followed hereafter, as it is quicker and easier than that involving dissections of glands followed by 30 minutes' exposure to ultra-violet radiation.

A second exception was the method of classifying the degree of infection in the series of fowls receiving inoculations of infected blood. In this series we have followed a new method recently described by Beckman (1941). Using his method we have classified infections as follows :—

Negative	= No parasites seen in 3 minutes.
+	= Less than 20 parasites seen in 3 minutes.
++	= Twenty organisms counted in from 2 to 3 minutes.
+++	= Twenty organisms counted in from 1 to 2 minutes.
++++	= Twenty organisms counted in from 30 to 60 seconds.
+++++	= Twenty organisms counted in 30 seconds or less.

Beckman's method was derived from experimental studies and, so far as we have tested it, we believe it is as useful as, and somewhat less time-consuming than, the technique which we employed previously, as described in the last paper (Mulligan, Russell and Mohan, 1941).

Mosquito-borne infections were obtained by allowing two mosquitoes (later dissected to prove presence of sporozoites in the glands) to feed to repletion on the breast of each fowl. In previous experiments sometimes only one mosquito was used, with occasional negative results in control. When two mosquitoes were used there were no negative results.

Infections with trophozoites were produced by intravenous injections of 0.25 c.c. of citrated blood from fowl 254, from which 10 c.c. of blood was taken from the heart while the bird was lightly under chloroform. This fowl at the time had an infection classed as ++; it had been infected by blood inoculation a week earlier from fowl 250. Fowl 254 made a spontaneous recovery and was negative eight days after its blood had been taken. The entire 10 c.c. of blood from this fowl was kept in the syringe into which it had been drawn, and all of the experimental and control fowls received, in rapid succession, a measured dose from this sample of citrated blood. Fowl 258 was given 0.25 c.c. of this same blood one hour after it had been drawn, half an hour after the last fowl in the series had received its injection. Fowl 258 became positive four days later and made a spontaneous recovery, after an infection that did not exceed ++.



## RESULTS.

Tables II and III have been prepared to illustrate the specific agglutinogenic properties of sporozoites by summarizing previous results and those reported here for the first time. It will be seen (Table II) that of 28 normal fowls examined, none had an agglutinating titre against sporozoites of *P. gallinaceum* of more than 1/256. In Table III it will be noted that no fowls receiving five or more injections of inactivated sporozoites failed to show titres of under 1/16,384, and some had agglutination titres up to 1/262,144.

In Table IV are shown results, not previously reported, of infections in normal fowls bitten by two infective mosquitoes, and in Table V these results have been added to previously reported results in fowls either normal or having agglutination titres of 1/16,384 or less. It will be seen that among the 35 fowls in this entire series, bitten by one or by two infective mosquitoes, there were 18 deaths, a mortality rate of 51.4 per cent. The percentage of spontaneous recoveries was 48.6, and the average prepatent period was 10.0 days.

TABLE I.

*Vaccinated fowls with agglutination titre 1/32,768 or higher, fed upon by two infective mosquitoes (P. gallinaceum).*

Serial number.	Prepatent period (days).	Peak of infection.	Result.	Agglutination titre.
165	7	+	S.R.	1/32,768
166	6	++++	D	1/65,536
171	11	++	S.R.	1/262,144
173	10	+	S.R.	1/131,072
176	9	+	S.R.	1/65,536
177	9	+	S.R.	1/131,072
185	9	+	S.R.	1/262,144

Notes.—These fowls have not been reported previously.  
D = died. S.R. = spontaneous recovery.

TABLE II.

*Agglutination titres of normal fowls against sporozoites of P. gallinaceum.*

Description.	Fowls tested.	Number with titres of					
		1/8	1/16	1/32	1/64	1/128	1/256
Number ..	28	2	5	3	5	8	5
Per cent of all examined ..	—	7.1	17.9	10.7	17.9	28.6	17.9

Note.—One normal fowl (No. 195) fed on by two infected mosquitoes failed to become infected. Twenty days afterwards it had an agglutinating titre of 1/4,000. It was then infected by bites of two more mosquitoes and recovered spontaneously after a mild infection.

Eleven of the above fowls have not been reported previously.



TABLE III.

*Résumé of experiments showing specific agglutinogenic properties of inactivated sporozoites of P. gallinaceum.*

Description.	FOWLS RECEIVING THREE INJECTIONS (i).		FOWLS RECEIVING FOUR INJECTIONS (ii).		FOWLS RECEIVING FIVE INJECTIONS (iii).		FOWLS RECEIVING SEVEN INJECTIONS (iv).	
	Number.	Per cent.	Number.	Per cent.	Number.	Per cent.	Number.	Per cent.
Agglutination titre								
1/512 ..	1 (v)	16.7	..	..	..	..	..	..
1/1,024 ..	..	..	1 (vii)	20.0	..	..	..	..
1/2,048 ..	..	..	1	20.0	..	..	..	..
1/4,096 ..	2	33.3	..	..	..	..	..	..
1/8,192 ..	1	16.7	1	20.0	..	..	..	..
1/16,384 ..	1	16.7	2	40.0	3	15.0	..	..
1/32,768 ..	..	..	..	..	6	30.0	..	..
1/65,536 ..	..	..	..	..	5	25.0	1	33.3
1/131,072 ..	..	..	..	..	4	20.0	1	33.3
1/262,144 ..	1 (vi)	16.7	..	..	2 (viii)	10.0	1 (ix)	33.3
TOTAL ..	6	—	5	—	20	—	3	—

Notes.—Nineteen of above 34 fowls reported previously.

(i)	Received total of	from 60 to 120	dissections.	Sporozoites killed by ultra-violet radiation.
(ii)	"	"	" 70 " 190	"
(iii)	"	"	" 190 " 200	"
(iv)	"	"	" 180 " 220	"
(v)	"	"	" 120 dissections.	"
(vi)	"	"	" 60	"
(vii)	"	"	" 70	"
(viii)	"	"	" 200	"
(ix)	"	"	" 180	"

Referring back to Table I, results were shown, not previously reported, of infections in fowls that had received injections of inactivated sporozoites and had developed agglutination titres of 1/32,768 or higher. In Table VI these data have been added to results previously reported. It will be seen that among 13 fowls in the entire series the mortality rate was 7.7 per cent, with 92.2 per cent spontaneous recoveries. In the one case where death ensued, the degree of infection was + + + +. In one other case it was + +, but in the remaining 11 cases it did not exceed +. In the series of 35 normal fowls



(Table V), 19 had infections of +++ or ++++ degree and only 9 failed to exceed +.

In other words, there was clear evidence of partial protection against sporozoite-borne infection in those fowls having an agglutination titre of 1/32,768 or higher against homologous sporozoites.

TABLE IV.

*Normal fowls fed on by two infected mosquitoes (P. gallinaceum).*

Serial number.	Prepatent period (days).	Peak of infection.	Result.	REMARKS.
195	9	++	S.R.	Normal.
196	10	++++	D	"
197	7	+	S.R.	"
198	7	+++	D	"
215	14	+	S.R.	"
219	8	+	S.R.	"
220	8	+	S.R.	"

Notes.—These fowls have not been reported previously.  
D = died. S.R. = spontaneous recovery.

TABLE V.

*Results when one or two infected mosquitoes fed on fowls either normal or having agglutinating titre, against homologous sporozoites, of 1/16,384 or less (P. gallinaceum).*

Description.	Number of fowls.	Total of prepatent periods (days).	RESULT.	
			Number died.	Number S.R.
Normal fowls previously reported ..	22	223	13	9
Fowls with agglutinating titre 1/16,384 or less previously reported.	6	65	3	3
Normal fowls reported herewith (Table IV)	7	63	2	5
Totals .. ..	35	351	18	17
Average or percentage .. ..	—	10.0	51.4	48.6

Notes.—Normal fowls previously reported, Nos. 59, 61, 63, 109, 110, 112, 117, 122, 131, 134, 140, 141, 142, 143, 144, 145, 146, 147, 158, 159, 160, 164.  
Fowls with titre 1/16,384 or less previously reported, Nos. 79, 80, 81, 82, 121, 123.  
S.R. = spontaneous recovery.



TABLE VI.

*Results when one or two infected mosquitoes fed on fowls having agglutinating titre, against homologous sporozoites of 1/32,768 or higher (P. gallinaceum).*

Description.	Number of fowls.	Total of prepatent periods (days).	RESULT.	
			Number died.	Number S.R.
Fowls previously reported .. ..	6	60	0	6
Fowls reported herewith (Table I) ..	7	80	1	6
Total .. .. .	13	140	1	12
Average or per cent .. ..	—	10·8	7·7	92·2

Notes.—Fowls previously reported, Nos. 124, 125, 126, 127, 128, 150.  
S.R. = spontaneous recovery.

#### TROPHOZOITE INFECTIONS.

Some indications as to whether or not this partial immunity following sporozoite vaccination, and observed to be effective against homologous sporozoites, would be equally effective against blood inoculations of homologous trophozoites, was obtained in the experiment analysed in Tables VII and VIII. Fowls 201 to 204, 206, and 208 to 211 were given weekly intravenous injections of 40 salivary gland dissections of *P. gallinaceum*, inactivated by 30 minutes' exposure to ultra-violet light, in the same manner as in preceding experiments reported above (excluding Nos. 176, 177 and 185 not in this series but described above). Eight days after the last injection, blood was taken for testing the agglutination titre against homologous sporozoites, with the results shown in Table VII. All but fowl 209 showed titres of 1/32,768 or higher, in one case (206) of 1/262,144. The agglutination titres of normal fowls 221, 223, and 226 to 230 were also tested and none was found higher than 1/256 (Table VIII).

All of these vaccinated and normal fowls (201 to 204, 206, 208 to 211, 221, 223, 226 to 230) were then given an intravenous injection of 0·25 c.c. of infected blood from fowl 254, as described above. All injections were made from the same sample, and all fowls were examined daily thereafter until death or spontaneous recovery.

The results of these trophozoite inoculations are shown in Tables VII and VIII. It will be seen that the mortality in the normal fowls was 28·6 per cent and in the vaccinated fowls 44·4 per cent. But if fowl 209, which had a titre of less than 1/32,000 (apparently a dividing line in the sporozoite infection series), is transferred to the normal group, then the mortality in the vaccinated and normal groups was the same, 37·5 per cent. There were no significant differences in the two groups, considering only birds that recovered spontaneously, in the average number of days positive or the average number of days



when the degree of infection was ++++ or +++++. The prepatent (incubation) period was 3.6 days in the normal birds and 5.0 in the other group.

It seems clear from these results that agglutination titres in vaccinated fowls that would indicate some degree of protection against infection by homologous sporozoites did not indicate any protection at all against infection by homologous trophozoites.

TABLE VII.

*Results following blood inoculation of infective trophozoites of P. gallinaceum in fowls vaccinated by injections of killed homologous sporozoites.*

Fowl number.	Highest agglutination titre.	Prepatent period (days).	Total number of days positive.	Total number of days +++++ or +++++.	Result.
201	1/131,072	8	8	6	D
202	1/65,536	3	8	6	D
203	1/32,768	4	13	4	S.R.
204	1/65,536	1	15	7	S.R.
206	1/262,144	3	4	1	D
208	1/65,536	3	15	10	S.R.
209	1/16,384	6	9	5	D
210	1/32,768	8	8	1	S.R.
211	1/32,768	9	12	9	S.R.

Notes.—D = died. S.R. = spontaneous recovery.

Mortality	..	..	..	..	44.4 per cent.
Prepatent period average in days	..	..	..	..	5.0 "
Average number of days positive in those that recovered	..	..	..	..	12.6 "
Average number of days +++++ or +++++ in those that recovered	..	..	..	..	6.2 "
Mortality omitting No. 209 (with titre less than 1/32,768)	..	..	..	..	37.5 per cent.

TABLE VIII.

*Results following blood inoculation of infective trophozoites in normal fowls.*

Fowl number.	Highest agglutination titre.	Prepatent period (days).	Total number of days positive.	Total number of days +++++ or +++++.	Result.
221	1/256	4	4	2	D
223	1/256	3	15	9	S.R.
226	1/128	4	12	4	S.R.
227	1/128	5	17	11	S.R.
228	1/128	3	9	4	S.R.
229	1/16	3	5	2	D
230	1/32	3	9	5	S.R.

Notes.—D = died. S.R. = spontaneous recovery.

Mortality	..	..	..	..	28.6 per cent.
Prepatent period average in days	..	..	..	..	3.6 "
Average number of days positive in those that recovered	..	..	..	..	12.4 "
Average number of days +++++ or +++++ in those that recovered	..	..	..	..	6.6 "
Mortality including No. 209 (with titre less than 1/32,768)	..	..	..	..	37.5 per cent.



## DISCUSSION.

It is obvious that, under the conditions of the experiments reported above, sporozoite vaccination of fowls gave partial protection against mosquito-borne infections of homologous sporozoites, but not against intravenous injections of homologous trophozoites. This suggests that trophozoites and sporozoites are not immunologically identical. But it must be pointed out that perhaps the infective doses of sporozoites were quantitatively so much less than those of trophozoites that partial immunity against the latter was masked. We do not know how many sporozoites a mosquito injects at one feeding, although we have planned a study of this point. Also it might have been better for comparative purposes, if the blood injections had been given intramuscularly, as are normal mosquito-injected sporozoites. This was not done because previous experience indicated that more consistent results from injections of infected blood are seen in normal fowls when the intravenous rather than the intramuscular route is used.

The question of the immunological identity of sporozoites and trophozoites has never been answered. Boyd and Kitchen (1936) and Boyd, Stratman-Thomas and Kitchen (1936) suggested that acquired antiparasitic immunity observed in certain malarial infections is directed, mainly at least, against trophozoites. Sinton (1940) inclined towards the same view, although his experiments with *P. ovale* indicated, as he pointed out, that possibly a true immunity against sporozoites could be developed. Wolfson and Causey (1939) concluded, as a result of experiments in birds with two strains of *P. cathe-merium*, that immunity to superinfection is probably effective against sporozoites as well as trophozoites. Sinton (*loc. cit.*) attributed a greater 'aggressivity' to sporozoites than to trophozoites of *P. ovale*. He noted that immunity, developed as a result of infection induced by sporozoites, was more effective than immunity resulting from infection following blood inoculation of trophozoites.

However, in the experiments reported above, a degree of immunity that clearly modified infection following sporozoite inoculation did not appear to have any effect at all on infection following the inoculation of a rather small quantity of blood containing relatively few trophozoites.

## SUMMARY AND CONCLUSIONS.

1. Previous work with domestic fowls, indicating that repeated injections of large numbers of inactivated sporozoites of *P. gallinaceum* will cause a considerable rise in the agglutination titre of the serum against homologous sporozoites in every case, is confirmed.

2. A method of preparing inactivated sporozoites for injection by grinding and drying thoraces of infected mosquitoes, is described as an alternative to the use of ultra-violet radiation.

3. The fact that, while fowls vaccinated in this way are susceptible to mosquito-borne infection with the homologous strain of *P. gallinaceum*, yet those fowls having an agglutination titre of 1/32,000 or higher will develop only mild infection, is confirmed. The mortality rate in such fowls was 7.7 per cent, as contrasted with a rate of 51.4 per cent in fowls normal or having an agglutination titre of 1/16,384 or less.



4. In a series of fowls there was no evidence that the same sporozoite vaccination that partially protected against infections by homologous sporozoites gave any protection at all against intravenous injections of blood containing homologous trophozoites.

# REFERENCES.

- BECKMAN, H. (1941) .. .. An experimentally derived method for determining the degree of infection in avian malaria. *Amer. J. Trop. Med.*, **21**, 1, pp. 151-157.
- BOYD, M. F., and KITCHEN, S. F. (1936). Is the acquired homologous immunity to *P. vivax* equally effective against sporozoites and trophozoites? *Ibid.*, **16**, 3, pp. 317-322.
- BOYD, M. F., STRATMAN-THOMAS, W. K., and KITCHEN, S. F. (1936). On acquired immunity to *Plasmodium falciparum*. *Ibid.*, **16**, 2, pp. 139-145.
- MULLIGAN, H. W., RUSSELL, P. F., and MOHAN, B. N. (1940). Specific agglutination of sporozoites. *J. Mal. Inst. Ind.*, **3**, 4, pp. 513-524.
- Idem* (1941). Active immunization of fowls against *Plasmodium gallinaceum* by injections of killed homologous sporozoites. *Ibid.*, **4**, 1, pp. 25-34.
- RUSSELL, P. F., MULLIGAN, H. W., and MOHAN, B. N. (1941). Specific agglutinogenic properties of inactivated sporozoites of *P. gallinaceum*. *Ibid.*, **4**, 1, pp. 15-24.
- SINTON, J. A. (1940) .. .. Studies of infections with *Plasmodium ovale*. IV. The efficacy and nature of the immunity acquired as a result of infections induced by sporozoite inoculations as compared with those by trophozoite infections. *Trans. Roy. Soc. Trop. Med. Hyg.*, **33**, 4, pp. 439-446.
- WOLFSON, F., and CAUSEY, O. R. (1939). Immunity to superinfection with sporozoites in two strains of *Plasmodium catheherium*. *J. Parasit.*, **25**, 6, pp. 510-511.







## ON THE INTERMITTENT IRRIGATION OF RICEFIELDS TO CONTROL MALARIA IN SOUTH INDIA\*.

BY

PAUL F. RUSSELL,  
FRED. W. KNIPE,

AND

H. RAMANATHA RAO.

[December 6, 1941.]

### INTRODUCTION.

THIS paper reports some studies, extending over 3 years, on the intermittent irrigation of ricefields in Pattukkottai Taluk, Tanjore District, Madras, as a measure for malaria control. These experiments resulted in effective control of *Anopheles* breeding, with no increase in weed growth, and with little or no effect on the yield or quality of grain or the weight of straw.

Not many such experiments have been recorded, and most have been limited in scope and design, especially as regards the effect of this procedure on yield of grain and straw. But, recently, Hill and Cambournac (1941) have reported an extensive and well-controlled study in Portugal, where, during the years 1935-1939, they observed intermittent irrigation of ricefields for malaria control on an increasing scale. They stated definitely: 'It has been found with regularity that this periodic drying of the ricefields greatly reduces the number of mosquito larvæ, decreases the amount of water consumed and, with most varieties of rice tested, increases the total yield without detriment to quality'. Furthermore, they concluded: 'This method of irrigation is not incompatible with good agricultural practice. In fact, we have yet to find any points where the two are divergent'.

The cycle practised in Portugal consisted of 10 days of wet fields followed by 7 days drying or dry. It required 2 or 3 days for water to drain off and about 4 days for complete drying. As the authors pointed out, the time necessary for wetting, as well as for draining and drying, will vary with size of plots, character of soil, temperature and humidity, height of rice, and other factors.

---

\*The experiments reported in this paper were carried out under the auspices, and with the support, of the International Health Division of the Rockefeller Foundation. Grateful acknowledgment is made to T. Ramachandra Rao, Entomologist, for his assistance.



In measurements extending over 3 years, they found that there was an average saving of over 6,000 tons of water per hectare (2.47 acres) of land in the intermittently irrigated plots as compared with those continuously irrigated. On the basis of these studies the Government of Portugal passed a new law in 1938 which included a clause making intermittent irrigation obligatory when so ordered by the Malaria Service. The authors noted that the Malaria Service had not yet had to use this law, because an increasing number of farmers were asking for co-operation in starting intermittent irrigation.

Among other reports of intermittent irrigation of rice for malaria control may be mentioned those of Konsuloff (1922; 1933), Ananyan (1929; 1930), Enikolopov (1931), Prosolupov (1931), and Smalt (1937). There is also a statement (League of Nations, 1937) about successful results with this measure in Indo-China where rice was alternately irrigated for 3 days and allowed to dry for 3 days. This gave complete larval control and it was stated: 'From the agricultural point of view, the paddy harvested differed neither in quantity nor in quality from that simultaneously grown on neighbouring rice-fields which were always under water'.

Few observations on this measure of malaria control appear to have been made in India. However, the following statement appears in the Annual Report of the Agricultural Research Station, Aduturai (Tanjore Delta area), for 1938-39.

'It is again found that it is not necessary to allow more than 1½ to 2 inches of water in the fields at any time and that no harm results if no standing water is allowed for 2 days in between two waterings. This system of intermittent irrigation will prevent mosquito breeding in paddy fields, as it has been established that without free water on the surface, mosquito larvæ cannot survive, particularly so when the watering interval is 5 to 6 days, which nearly corresponds to the larval period. Apart from this the economic use of irrigation water from the Mettur system, which has cost the taxpayer nearly seven crores of rupees to build, is a matter to be always borne in mind by every user.'

In Nilakkottai and adjoining taluks of Madura District, rice is grown in rotation with garden crops and irrigated from wells, perforce with a sort of intermittent irrigation. Frequently, there is no standing water in the ricefields, yet the crop does well.

In Mysore, according to the State Director of Agriculture, there has been found a high yielding variety of rice (Bangarakaddy or S705) which can be grown in sandy soils under intermittent irrigation. Indeed, it is reported that this rice can be grown without bunding and flooding the fields, and with only one period of wetting each week to 10 days.

In South Arcot District, where large tanks and wells are the main sources of irrigation, the ryots frequently find it impossible to keep water in their fields continuously. The *Dalwa* ('late second') crop of the Godavari Delta and the *Pishanam* ('late second') and *Manavari* ('early summer') crops of the southern districts of Madras, owing to the lateness of the season, receive far less water than the main crop, with a period up to 10 days between two wet periods. The ryots refer to dry periods as *semi-dry* and believe that such drying does the rice crop no harm. On the whole, in areas where the water supply is somewhat limited, and where the soil is fairly well drained, it is common for ricefields to remain dry enough from time to time to inhibit *Anopheles* breeding. If such dry periods could be regularized as to duration and periodicity, practical larval control would be obtained.



### CHARACTERISTICS OF PATTUKKOTTAI TALUK.

Pattukkottai Taluk has an area of some 433,500 acres, of which about 120,000 (65 per cent of cultivated land) are under wet rice cultivation. The taluk is flat and free from forest. Prior to the opening of the Cauvery-Mettur Irrigation Scheme in 1933, the countryside was dry and was never malarious. Since the inauguration of this system of canal irrigation, the area has been practically inundated each year from mid-June to the following mid-January, an ever increasing acreage has been devoted to rice, and malaria has become endemic.

The Pattukkottai climate is moderately dry tropical, with an average annual rainfall of about 44 inches, and an average of about 50 days a year on which some rain falls. The mean temperature is about 28°C. (82.4°F.), and average relative humidity about 74.6 per cent. The lowest mean temperature recorded in 1937-1939 was 24.2°C. in December 1938; the highest 30.5°C. in June 1937, May 1938 and June 1939. The lowest mean relative humidity was 60.8 per cent in June 1938; the highest, 81.7 in November 1937. The chief rains come with the north-east monsoon, usually in November, when almost daily rains may be the rule. There may be showers in April and May. The points to be noted especially in regard to climate are: (1) except during the north-east monsoon in November, conditions favour rapid drying of water in rice-fields; (2) weather conditions are never unsuitable to mosquito activity. In no month is the mean relative humidity below 60 per cent or the mean temperature above 31°C. (87.8°F.) or below 24°C. (75.2°F.) (Russell and T. R. Rao, 1940).

### CHARACTERISTICS OF RICE CULTURE IN PATTUKKOTTAI.

As a rule, in Pattukkottai Taluk, only one rice crop is grown, the season, including the nursery stage, being from mid-July to mid-January. Where there are two crops, the first grows from mid-June to October, and the second from mid-October to February. Rice in Pattukkottai is usually sown in nurseries and then transplanted. The nursery period is from 30 to 45 days. While nurseries are growing, fallow fields are wetted and any available manure, such as ashes, cattle-shed sweepings, or ground-nut (pea-nut) shells is applied. Just prior to transplanting the rice, fields are ploughed three or four times and are well puddled and levelled. Newly transplanted fields are kept slightly wet for a week, while the plants strike root. Thereafter, they are continuously irrigated from small field channels which take off from larger ones, until a fortnight before the harvest, when they are drained and allowed to dry. Most ricefields remain wet fallow for 2 months after the onset of irrigation, and are not ploughed, puddled, and planted until September.

This preliminary phase of wet fallow fields or of young rice in Pattukkottai Taluk is the time of most intense breeding of *A. culicifacies*, the malaria vector, in the paddies and channels. There is an enormous output of this species from the very great acreage of ricefields, and it reaches a peak in late August or early September. It is not surprising that there follows in October and November an annual seasonal epidemic of malaria.

Breeding of *A. culicifacies* continues in the fields until the rice plants stand about a foot high, when there is a striking diminution in density. The non-carrying species *A. pallidus* and *A. hyrcanus* appear at this stage. Soon thereafter very few *A. culicifacies* can be found in the paddies, although breeding continues, at a lower density than before, in the channels.



In the contiguous Tanjore and Papanasam taluks, rice cultivation has been carried on for centuries, whereas in Pattukkottai it has become prevalent only since 1933-34. Two crops are the rule in the former taluks, so that the time between first wetting of fields and planting is very short, sometimes being only a week. Moreover, there is everywhere evidence of an almost uniform time-table of agricultural procedure. The result has been that, although *A. culicifacies* is found in ricefields and channels of those areas, the species never attains a density comparable to that seen in Pattukkottai. The fact that there are fewer channels is also important. The reason is that irrigation in Tanjore and Papanasam, as throughout the Delta, is by inundation from the river, whereas, in Pattukkottai, it is 'contour irrigation' by a complex system of canals and channels.

Unfortunately in Pattukkottai, irrigation has brought with it malaria, and, since 1933, the spleen rate has risen to an average of over 40 per cent, in some villages over 70 per cent. There seems no doubt that ricefields, irrigation canals, and field channels play a large part in generating the mosquitoes that transmit malaria in this area. The epidemiology of malaria in Pattukkottai has been discussed by Russell, Menon and Rao (1938), the malariogenic effects of this irrigation system by Russell (1938), natural mosquito infections by Russell and T. R. Rao (*loc. cit.*), and the anophelines of ricefields by Russell and H. R. Rao (1940).

## INTERMITTENT IRRIGATION.

### EXPERIMENT A IN CONTROLLED RANDOM PLOTS.

In order to test intermittent irrigation of ricefields as regards mosquito control and yield of grain and straw under controlled experimental conditions, some land  $2\frac{1}{2}$  acres in area (600 feet by 170 feet) was rented during the years 1938-1941. This level and uniform farm had been used for growing ground-nuts until 1937, when the first rice was planted. As is usual in this taluk, the sandy loam soil was deficient in organic matter, and its ability to retain moisture was low.

Twenty plots, each of  $1/40$  acre, measuring  $60\frac{1}{2} \times 18$  feet, were laid out in a row (Chart). Actually, the plots were made uniformly larger so that each had a one-foot 'outskirt', the rice from which could be discarded when harvesting the crop for weighing. Along the northern edge was the main irrigation supply channel. The plots were separated one from another by seepage drains placed at lower elevations than the plots themselves. All seepage drains led into a single drainage channel running along the southern margin. This method of drainage precluded any possibility of seepage between plots (Plate XXXII, figs. 1, 2; Plate XXXIII, fig. 3).

The twenty plots were laid out in four blocks, each block having five uniform plots. In these plots, the effect of four types of intermittent irrigation was tested. Four plots in each block were kept 5 days wet and then 1, 2, 3, or 4 days dry respectively, so that the total cycles were 6, 7, 8, and 9 days. The fifth plot (experimental control) was continuously irrigated. The plots were selected at random in each block, the four blocks permitting four replications. A paris green trap was installed at the head of the incoming water channel, so that no *Anopheles* larvæ could enter the experimental area (Russell and H. R. Rao, *loc. cit.*, (Plate XXXVIII, fig. 10).



PLATE XXXII.

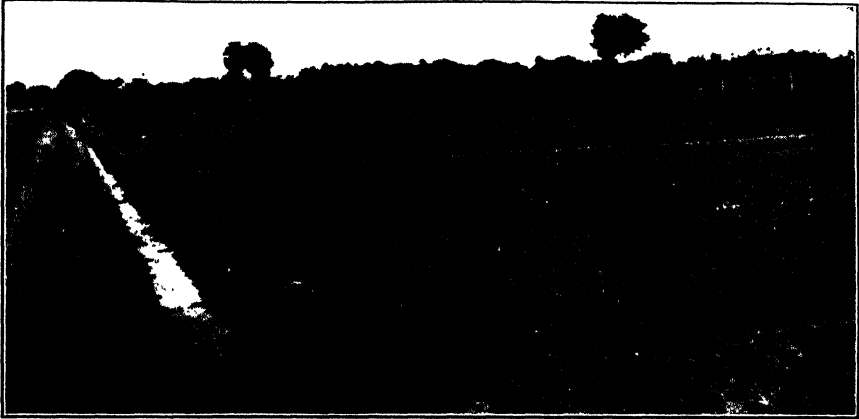


Fig. 1. Plots in Experiment A, ploughed and puddled, ready for trans-plantation of seedlings (looking north-east).



Fig. 2. Plots in Experiment A, when rice was almost half grown (looking north-west).



*PLATE XXXIII.*



Fig 3. Plots in Experiment A after harvest (looking north-east).

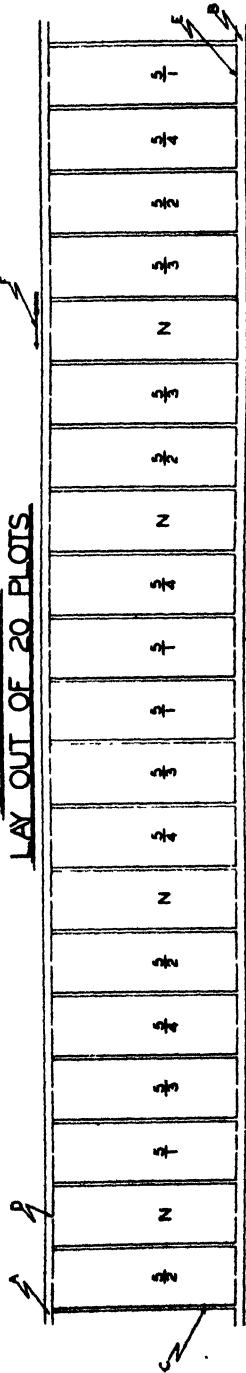


Fig. 4. Winnowing rice preparatory to weighing crop from each plot (Experiment A).



CHART.

INTERMITTENT IRRIGATION OF RICE  
EXPERIMENT A  
LAY OUT OF 20 PLOTS



- A FIELD SUPPLY CHANNEL
- B OUTFLOW CHANNEL
- C SEEPAGE DRAIN
- D INLET FOR EACH PLOT
- E OUTLET FOR EACH PLOT
- F FLOW DIRECTION

CONTINUOUS IRRIGATION		IRRIGATION		DAYS WET		DAYS DRY	
N	$\frac{1}{2}$	INTERMITTENT	IRRIGATION	5	-DO-	2	-DO-
$\frac{1}{2}$	$\frac{1}{2}$	-DO-	-DO-	5	-DO-	3	-DO-
$\frac{1}{2}$	$\frac{1}{2}$	-DO-	-DO-	5	-DO-	4	-DO-
$\frac{1}{2}$	$\frac{1}{2}$	-DO-	-DO-	5	-DO-		



For seed, we chose the variety of rice commonest in the region. It is called Adt. 10 (*Kattasambalai*) and matures in about 166 days. There are about half a million acres of this variety under cultivation in Tanjore District.

Anopheles mosquitoes develop rapidly in this area during the months of June to September, and it was not possible to allow a longer period than 5 wet days if one were to interrupt the cycle of *A. culicifacies*, between egg and adult. Hence we chose this maximum period of 5 wet days. As to dry days, the soil had such low moisture retentivity that a 4-day dry period seemed about the maximum that could be tolerated without danger of soil-cracks or clod formation, which might damage the rice.

It must be emphasized that all the plots received uniform treatment, except as regards the irrigation water. During the wet days, water was supplied to keep the level at a constant depth of four inches, which is the average for the taluk. At the end of 5 days, an outlet pipe was opened and in 30 minutes the plot was drained, except for small pools in the inevitable depressions. These pools, as a rule, did not all become completely dry in 1 day, so that the 5 days wet : 1-day dry cycle did not give effective mosquito control. Contrary to the usual behaviour of small fishes, larvæ showed no tendency to try to collect in residual pools or to escape with the surface run off.

In the plots 2 days dry, the soil surface lost its water of saturation, so that there was no visible surface film. This gave nearly complete larval control, except in an occasional small pool.

In the plots 3 and 4 days dry, water of saturation had disappeared and there was complete larval control. There was still no cracking and the sub-surface was moist.

The results of larval collections are shown in Table I.

#### METHOD OF CULTIVATION.

All plots had four ploughings, followed by a light spade (*manimatty*) digging, uniformly carried out. The plots were then puddled and made ready for transplanting of rice. No manure was used during the first season, but in the second and third, as soon as water was available, we sowed in each plot two pounds of *Dhaincha* (*Sesbania aculeata*, Leguminosæ) for green manure. This was later ploughed under, and the fields were prepared as already described.

The Government Agricultural Research Station in Pattukkottai supplied 45-day old seedlings for our first season's experiment. We developed our own seedlings for the second and third seasons.

Great care was taken to obtain uniformity of planting, and experienced labour was used. In accordance with local custom, all plots were kept nearly dry for 5 days after transplanting.

#### RESULTS OF EXPERIMENT A.

Each season after the crop had been growing for about a month, we measured the height of plants at 20 points in each plot and obtained an average as an index. The results are shown in Table II, and it appears that there were no significant differences.

At the same time an average tiller count was made. These tillers are side branches of the rice plant, and their number gives some indication of probable yield of grain. Tiller indices are tabulated in Table III, where it will be seen



TABLE I.  
Effect of intermittent irrigation on anophelines breeding in ricefields, Pattukkottai studies, 1938-1941.

TYPE OF IRRIGATION.																				
Description.	5 days wet—1 day dry.					5 days wet—2 days dry.					5 days wet—3 days dry.					5 days wet—4 days dry.				
	I	II	III	IV	P	I	II	III	IV	P	I	II	III	IV	P	I	II	III	IV	P
Before and after rains.	1	252	569	84	71	2	16	..	3	..	..	6	..	..	..	16	8	2	..	..
	2	696	593	321	143	5	314	36	2	4	..	630	8	1	1	402	196	6	..	..
	3	402	463	383	110	17	1,610	1,018	16	8	..	502	354	24	..	921	688	64	8	..
	4	885	611	433	381	28	1,437	983	864	2	..	987	1,012	951	43	1,406	722	406	7	..
	5	594	873	599	313	6	832	892	981	431	..	611	1,414	881	123	493	670	783	245	..
During rains.	1	101	69	167	48	2	145	44	32	3	1	28	36	18	14	26	12	23	1	1
	2	293	418	146	96	20	48	79	16	28	2	201	16	34	13	106	33	18	2	..
	3	473	213	64	35	7	364	684	84	25	..	149	173	33	42	128	164	49	19	1
	4	121	343	48	65	16	103	371	301	64	8	221	312	111	8	106	125	137	24	2
	5	110	312	212	46	6	36	139	341	184	6	169	239	141	43	26	142	113	128	4



TABLE II.

*Intermittent irrigation of rice, height of crop in inches, Experiment A.*

Description.	Block.				Total.	Average.
	I	II	III	IV		
1938-39—1st season.						
Control .. ..	16.6	18.0	18.6	19.4	72.6	18.2
5 days wet—1 day dry ..	16.9	18.0	18.4	18.1	71.4	17.9
5 days wet—2 days dry ..	15.6	17.0	18.4	19.4	70.4	17.6
5 days wet—3 days dry ..	16.2	16.6	18.8	17.4	69.0	17.3
5 days wet—4 days dry ..	16.4	16.8	20.4	18.6	72.2	18.1
1939-40—2nd season.						
Control .. ..	27.3	28.1	29.4	31.2	116.0	29.0
5 days wet—1 day dry ..	29.9	28.8	27.2	31.9	117.8	29.5
5 days wet—2 days dry ..	28.5	31.4	28.9	30.1	118.9	29.7
5 days wet—3 days dry ..	29.8	30.2	30.2	32.6	122.8	30.7
5 days wet—4 days dry ..	28.1	29.5	29.6	30.2	117.4	29.4
1940-41—3rd season.						
Control .. ..	29.3	28.9	28.5	31.3	118.0	29.5
5 days wet—1 day dry ..	28.5	28.8	28.8	30.8	116.9	29.2
5 days wet—2 days dry ..	28.6	29.0	29.9	32.8	120.3	30.1
5 days wet—3 days dry ..	29.1	29.2	30.9	29.9	119.1	29.8
5 days wet—4 days dry ..	26.8	29.2	29.7	31.4	117.1	29.3

that there were no significant differences between control and intermittently irrigated plots.

About six weeks after transplanting we weeded all plots, weighing the green weeds uniformly. The results are shown in Table IV. It is clear that the intermittent irrigation of ricefields did not tend to increase the growth of weeds. We can also add that it did not change the species of weeds.

The first season was abnormal in that the north-east monsoon failed and also the supply of canal water was highly irregular. From time to time we had to use well water lifted by a centrifugal pump. There was slight damage to the rice by the mealy bug (*Ripersia oryzae*), but it did not appear that this pest made any discrimination between the control and experimental plots. Another and much more serious difficulty during the first season was 'blast' disease,



caused by the fungus *Piricularia oryzae*, which was widespread throughout the countryside, especially after the rice had flowered. This fungus appears to be associated with drought conditions, and it seemed to attack our intermittent plots to a greater extent than the controls. This was noticed after the rice had come to flower, but not before.

TABLE III.

*Intermittent irrigation of rice, numbers of tillers, Experiment A.*

Description.	Block.				Total.	Average.
	I	II	III	IV		
<i>1938-39—1st season.</i>						
Control .. ..	6.7	7.5	6.3	7.9	28.4	7.1
5 days wet—1 day dry ..	6.4	7.1	6.4	7.7	27.6	6.9
5 days wet—2 days dry ..	6.8	6.9	7.3	8.1	29.1	7.3
5 days wet—3 days dry ..	6.6	6.6	8.4	7.4	29.0	7.3
5 days wet—4 days dry ..	6.5	6.2	9.7	7.5	29.9	7.5
<i>1939-40—2nd season.</i>						
Control .. ..	4.5	4.9	5.0	4.9	19.3	4.8
5 days wet—1 day dry ..	5.0	5.4	4.9	4.5	19.8	5.0
5 days wet—2 days dry ..	4.7	4.6	5.2	4.5	19.0	4.8
5 days wet—3 days dry ..	5.2	4.8	5.6	5.9	21.5	5.4
5 days wet—4 days dry ..	4.9	4.3	4.3	4.9	18.4	4.6
<i>1940-41—3rd season.</i>						
Control .. ..	4.7	5.5	5.4	5.2	20.8	5.2
5 days wet—1 day dry ..	4.3	4.1	5.3	6.2	19.9	5.0
5 days wet—2 days dry ..	4.1	5.5	5.3	5.6	20.5	5.1
5 days wet—3 days dry ..	5.2	5.2	4.9	4.6	19.9	5.0
5 days wet—4 days dry ..	4.1	6.5	4.1	5.8	20.5	5.1

The second season had a more normal rainfall, with no failure of monsoon. But, early in November, a cyclone came overland from the Bay of Bengal and no less than eight inches of rain fell in one night. This caused widespread floods and submerged rice everywhere in the area. Some main canals were breached so that they carried no water for three weeks, and we had to rely on water pumped from the well. The floods did not seem to damage our rice, and the crops were not seriously attacked by any pests during the second season. The



TABLE IV.

*Intermittent irrigation of rice, weight of weeds in pounds, Experiment A.*

Description.	BLOCK.				Total.	Average.
	I	II	III	IV		
1938-39—1st season.						
Control .. ..	10.0	9.5	12.0	22.0	53.5	13.4
5 days wet—1 day dry ..	8.0	6.0	7.5	6.0	28.0	7.0
5 days wet—2 days dry ..	6.5	12.5	4.5	20.0	43.5	10.9
5 days wet—3 days dry ..	11.0	3.0	5.2	9.0	28.2	7.1
5 days wet—4 days dry ..	7.2	4.5	15.0	8.0	34.7	8.7
1939-40—2nd season.						
Control .. ..	0.3	0.3	6.0	12.0	18.6	4.7
5 days wet—1 day dry ..	0.3	2.0	2.0	11.0	15.3	3.8
5 days wet—2 days dry ..	1.0	0.3	6.0	12.0	19.3	4.8
5 days wet—3 days dry ..	0.5	0.5	2.3	7.0	10.3	2.6
5 days wet—4 days dry ..	1.0	0.8	2.0	8.5	12.3	3.1
1940-41—3rd season.						
Control .. ..	12.0	12.0	13.0	7.0	44.0	11.0
5 days wet—1 day dry ..	9.0	7.5	3.5	8.0	28.0	7.0
5 days wet—2 days dry ..	6.0	22.0	19.0	6.0	53.0	13.3
5 days wet—3 days dry ..	8.5	10.5	7.0	2.0	28.0	7.0
5 days wet—4 days dry ..	4.0	3.5	20.0	14.5	42.0	10.5

third season was fairly normal as regards rainfall, and there was no serious damage due to rice disease.

In the middle of the first and third seasons we applied a uniform amount of a commercial fertilizer (Nicifos) to all plots, at the rate of 50 pounds per acre.

In all three seasons a casual observer could not have detected gross differences between control and experimental plots, although in the first season careful examination towards harvest time showed that fungus disease had done more damage to the intermittent plots.

Great care and intimate supervision were exercised in harvesting, so that the weights of grain and straw for the individual plots could be determined accurately. Results are shown in Tables V and VI, the former giving weights of grain and the latter of straw (Plate XXXIII, fig. 4).



TABLE V.

*Intermittent irrigation of rice, weight of grain in pounds, Experiment A.*

Description.	Block.				Total.	Average.
	I	II	III	IV		
1938-39—1st season.*						
Control .. ..	71.4	64.6	81.9	88.8	306.7	76.7
5 days wet—1 day dry ..	51.1	63.4	64.5	95.2	274.2	68.6
5 days wet—2 days dry ..	35.4	48.8	64.7	83.9	232.8	58.2
5 days wet—3 days dry ..	56.8	51.6	73.3	79.5	261.2	65.3
5 days wet—4 days dry ..	32.4	33.2	62.3	57.0	184.9	46.2
1939-40—2nd season.						
Control .. ..	88.3	89.1	84.8	89.9	352.1	88.0
5 days wet—1 day dry ..	82.3	88.3	83.5	88.0	342.1	85.5
5 days wet—2 days dry ..	87.8	90.8	93.9	87.3	359.8	90.0
5 days wet—3 days dry ..	102.8	74.8	96.8	81.3	355.7	88.9
5 days wet—4 days dry ..	83.3	98.5	93.8	82.9	358.5	89.6
1940-41—3rd season.						
Control .. ..	77.2	73.5	72.7	81.0	304.4	76.1
5 days wet—1 day dry ..	70.5	72.3	74.3	75.0	301.1	75.3
5 days wet—2 days dry ..	69.1	71.0	80.8	80.9	301.8	75.5
5 days wet—3 days dry ..	79.2	81.9	79.3	71.9	312.3	78.1
5 days wet—4 days dry ..	74.4	83.6	78.9	73.2	310.1	77.5

\* An unusual season characterised by seasonal fungus disease in experimental plots.

The effect of fungus disease is clearly shown in Table V, first season, to have been greater in the intermittently irrigated plots as regards yield of grain. But there was no effect on straw. In the other two seasons there were no significant differences, except that weight of straw was greater as a rule in the intermittent plots.

#### SUPPLEMENTARY EXPERIMENT B.

In a supplementary Experiment B, we tested during the last two seasons the effect of 5-wet : 2-dry intermittency, continued only up to the flowering stage of the rice. The same strain of seed (Adt. 10) was used, and the same methods



TABLE VI.

*Intermittent irrigation of rice, weight of straw in pounds, Experiment A.*

Description.	BLOCK.				Total.	Average.
	I	II	III	IV		
1938-39—1st season.						
Control .. ..	117	115	112	154	498	125
5 days wet—1 day dry ..	125	119	119	137	500	125
5 days wet—2 days dry ..	101	121	157	151	530	133
5 days wet—3 days dry ..	106	106	139	138	489	122
5 days wet—4 days dry ..	100	120	124	131	475	119
1939-40—2nd season.						
Control .. ..	106	123	115	119	463	116
5 days wet—1 day dry ..	142	107	119	124	492	123
5 days wet—2 days dry ..	129	127	114	120	490	123
5 days wet—3 days dry ..	135	150	141	102	528	132
5 days wet—4 days dry ..	133	129	124	116	502	126
1940-41—3rd season.						
Control .. ..	124	115	111	139	489	122
5 days wet—1 day dry ..	129	106	112	128	475	119
5 days wet—2 days dry ..	103	121	125	130	479	120
5 days wet—3 days dry ..	123	129	122	127	501	125
5 days wet—4 days dry ..	116	125	119	136	496	124

of preparation and general management, as in the primary Experiment A, excepting that only four plots were used, two controls and two intermittently irrigated. These were laid out as follows : C E E C where C=control and E=experimental plots.

We had no rice disease in the course of this experiment and could detect no significant differences in any respect between the controls and the intermittent plots.

The results of Experiment B are shown in Table VII. Yields of grain were slightly higher in the intermittent plots each season than in the control plots.



TABLE VII.  
Supplementary Experiment B.

Description.	1939-1940.				1940-1941.			
	Control.		Intermittent.		Control.		Intermittent.	
	I	II	Average.	I	II	Average.	I	II
Weight of weeds in pounds.	5.5	3.0	4.3	5.0	3.5	4.3	36.0	33.5
Number of tillers . .	4.9	5.6	5.3	5.2	5.8	5.5	6.1	5.9
Height of crops in inches.	27.2	29.7	28.5	30.2	31.8	31.0	28.0	28.4
Weight of grain in pounds.	62.4	70.0	66.2	70.6	72.5	71.6	65.9	78.7
Weight of straw in pounds.	115	126	121	124	138	131	124	139
							130	132
								131

Note.—Intermittency was only of 5 wet : 2 dry variety. Same strain of rice used as in Experiment A. Intermittent irrigation stopped when rice came to flower.



TABLE VIII.  
*Supplementary Experiment C.*

Description.	Block.				
	I	II	III	IV	Average.
1939-40.					
Control					
Weight of weeds in pounds	1.0	1.5	1.2	2.0	1.4
Number of tillers ..	5.7	5.1	4.8	4.4	5.0
Height of crop in inches ..	34.2	32.0	30.6	28.2	31.3
Weight of grain in pounds	96.3	73.9	78.8	73.9	80.7
Weight of straw in pounds	149	124	127	107	127
Intermittent 5 wet : 2 dry					
Weight of weeds in pounds	5.0	1.2	1.0	3.0	2.6
Number of tillers ..	6.0	5.4	4.6	4.9	5.2
Height of crop in inches ..	32.0	31.3	29.1	29.4	30.5
Weight of grain in pounds	80.4	75.5	88.5	81.3	81.4
Weight of straw in pounds	129	147	124	121	130
1940-41.					
Control					
Weight of weeds in pounds	15.0	5.0	6.0	31.0	14.3
Number of tillers ..	4.8	5.8	6.2	6.2	5.8
Height of crop in inches ..	30.8	26.4	28.6	30.1	29.0
Weight of grain in pounds	65.4	78.4	69.7	72.1	71.4
Weight of straw in pounds	134	139	124	127	131
Intermittent 5 wet : 2 dry					
Weight of weeds in pounds	26.0	9.0	14.5	17.0	16.6
Number of tillers ..	4.2	5.1	6.0	6.8	5.5
Height of crop in inches ..	28.9	28.9	28.2	29.3	28.8
Weight of grain in pounds	*54.3	64.5	71.4	72.3	65.6
Weight of straw in pounds	107	141	142	126	129

\* Badly infested with mealy bugs.

Note.—Same technique as in Experiment A, but a different strain of rice.



### SUPPLEMENTARY EXPERIMENT C.

For the last two seasons in a supplementary Experiment C, we planted eight uniform plots in four blocks to test a strain of rice known as No. 11340, supplied by the Government Farm at Aduturai. This strain is said to be resistant to the 'blast' fungus disease. In these plots we carried intermittent irrigation of the 5 wet : 2 dry variety right through to the time of harvest. The treatment of the plots was similar to that in Experiment A. The plots were arranged as follows : C E E C C E E C, where C = control and E = experimental plots.

In the first season we had no rice disease in this experiment, but in the second season one of the intermittent plots suffered badly from mealy-bug infestation. We had no evidence that this was not a chance occurrence, as mealy bug was fairly common throughout the region during that season. Except that this diseased plot affected the yield of grain, and so lowered the average for intermittent plots, we saw no significant differences between the controls and the intermittent plots. The results are shown in Table VIII.

### COMPARISON OF NUTRITIVE QUALITIES OF RICE CROPS.

Samples of rice from normal and intermittent plots were sent to the Nutrition Research Laboratories at the Pasteur Institute in Coonoor, to be compared as regards nutritive qualities. Through the courtesy of the Director, Dr. W. R. Aykroyd, the following report was received :—

Description.			Sample 1 continuous irrigation.	Sample 2 intermittent irrigation.
Moisture	..	..	11.51 per cent	10.42 per cent
Protein	..	..	6.0 "	6.0 "
Fat	..	..	1.8 "	1.8 "
Mineral matter	..	..	1.0 "	1.2 "
Carbohydrate	..	..	79.7 "	80.4 "
Calcium (Ca)	..	..	0.017 "	0.017 "
Phosphorus (P)	..	..	0.180 "	0.205 "
Iron (Fe)	..	..	5.0 mg. "	5.7 mg. "
Calorific value per 100 grams			360	360
Vitamin B <sub>1</sub>	..	..	3.9 g./gram	4.2 g./gram

*Note by Dr. Aykroyd.*—'The result is quite clear. There is no appreciable difference in the two samples as regards their content of the above food factors, and hence in nutritive value.'



## VILLAGE EXPERIMENTS.

*Intermittent irrigation in the experimental plots over a 2-year period indicated that 2 days' drying in every 7 days was not harmful to rice, in yield, quality, or weight of straw. It, therefore, seemed expedient to broaden the experiment to include some village planting areas. Two communities were chosen: (1) Alivalam, an area where no previous malaria control of any kind had been undertaken, and (2) Senjayakollai, where we had carried out minor engineering works for 2 years.*

## 1. ALIVALAM.

Alivalam is a village of 125 dwellings with a population of 533, situated about 3 miles from Pattukkottai. The inhabitants had cultivated rice for many years, long before the Cauvery-Mettur canal irrigation scheme was introduced. The crops were formerly irrigated by water impounded in a large tank (reservoir with earthen banks), about half a mile from the village. Reports indicate that malaria did not exist in this village before the present canal irrigation system brought water, about 1934, yet the spleen rate was 70 per cent in 1937.

There were as many as 70 large and small borrowpits, and also tanks, wells, and three distributing channels within the control area, but the decision was made not to do such minor engineering as borrowpit filling during the first year. Other methods of control were to be tried in the borrowpits. But before the onset of the irrigation season the distributing channels were put in order. Two were quite easily dealt with by a little cleaning and deepening. The third and much the longer one, was in an extremely bad state of repair, so that an entirely new channel had to be excavated to take its place. Calco sliding head-gates were installed at each of the distributing channel sluices. The purpose of these gates was to give complete control of irrigation water at all times.

An agreement had to be entered into with the Revenue Collector to compensate villagers for any crop losses due to the new irrigation practice. This agreement was required before the irrigation and revenue authorities would allow the project to proceed. Even so, a few of the villagers were not co-operative at first and attempted to secure water during the early drying period. But one of us (H. R. R.), a malaria agronomist, was in daily attendance and in no case was a supply of water refused where there was evidence that the crop needed it. This was occasionally true in fields most distant from the sluice gates, during the first stages of rice growth. Once the confidence of the ryots was obtained, no further minor difficulties of this kind were encountered. The season progressed without incident and the crop appeared to be as good as in past years. No compensation was demanded or paid.

The programme of intermittent irrigation adopted provided  $4\frac{1}{2}$  wet days and  $2\frac{1}{2}$  dry days each week. This slight variation from the experimental plots was decided upon because the soil was somewhat heavier, containing more clay and consequently drying more slowly. Field and distribution channels were subject to the same programme.

This schedule was adhered to in so far as possible, and only minor variations were required. The fields dried, with few exceptions, during the  $2\frac{1}{2}$  days' drying period. This fact reduced the total mosquito population considerably during the early stages of rice growth, when larvæ of *A. culicifacies* would otherwise have been abundant in the fields. The field channels also dried out well. These were the main objectives of the intermittent irrigation experiment.



Fluctuation of water levels in the main channel was found to be ineffective for larval control. The gradient in the canal was so slight that rapid fluctuation of water levels was impossible. Larvæ could not be stranded under such circumstances. Automatic paris green distributors were then put in the canal and these proved successful. Larval development was practically stopped once the paris green distributors began to function properly. These automatic distributors were of the Russell-Eaton (1934) type, somewhat modified, but the paris green—powdered charcoal mixture—as originally recommended was used. The distributors were located at advantageous points along the canal, usually about 1,200 feet apart. They were operated twice weekly, one hopper full of larvicide (approx. 0.47 c. ft.) being sufficient for an eight-hour run. Complete larval control resulted.

Concurrently with the intermittent irrigation of the fields and channels certain other breeding places were dealt with as follows :—

*Borrowpits* : None was filled or drained. Instead, local measures such as any farmer might try, were used, the object being water pollution to control *A. culicifacies* breeding. Some pits were used for manure storage and others were contaminated with rotting cactus. Both measures were successful.

*Ponds* : An effort was made, with some success, to induce animals to use certain ponds as wallows. Where such ponds were used regularly, no breeding of *A. culicifacies* occurred.

*Wolffia* was transplanted to other ponds, and, for the most part, it grew very well. It was noted that *Wolffia* developed best in ponds that were slightly polluted. An interesting natural transplantation was noted. Animals, particularly hogs, sometimes used *Wolffia* ponds as wallows, and frequently these animals then went to another pond where they deposited enough *Wolffia* from their backs to start successful growth.

*Tanks* : These were stocked with *Gambusia* but without success. Predatory fish which had not been eliminated destroyed them. Breeding of larvæ occurred along the edges and was controlled by occasionally clearing the tank edge.

*Wells* : All within the area were chlorinated to kill any fishes present, after which *Gambusia* were introduced. Thereafter, wells ceased to be a menace.

## 2. SENJAYAKOLLAI.

The second village chosen was Senjayakollai, where there were nearly 400 acres of rice under cultivation. Here we had been carrying out various minor engineering measures for 2 years to control malaria, but had not dealt with ricefields. It therefore seemed logical to introduce intermittent irrigation. At first, the ryots objected to the scheme, and much persuasion had to be used to convince them that their interests were being taken into full account. At the end of the season it appeared that this intermittently irrigated rice area had produced as good a crop as any other in the district.

The procedure in Senjayakollai was similar to that employed in Alivalam. Calco gates were installed at each sluice in order to ensure complete control of water to individual field channels. Fields were watered for  $4\frac{1}{2}$  days and dried for  $2\frac{1}{2}$  days each week. As in Alivalam, a few cases of special need for water, early in the season, were dealt with by the agronomist, and in no case did a field suffer from lack of water. There was good larval control in fields and channels throughout the season.



Here, as in Alivalam, fluctuation in the main canal was tried without success in controlling breeding. Automatic distribution of paris green was then employed. Field channels dried well during the 2½ days. *Gambusia* were introduced into wells and controlled larval breeding. No unfilled borrowpits remained in Senjayakollai.

### DISCUSSION.

The foregoing report describes what amounted to nine separate experiments on which to base a discussion of intermittent irrigation, first, as a measure for controlling the breeding of the malaria vector, *A. culicifacies*, and second, as a method of watering the rice crop. In all experiments the evidence indicated that intermittent irrigation cycles of 5 wet followed by 2, 3, or 4 dry days, in the climate and *with the soil conditions* of Pattukkottai, effectively checked breeding of *A. culicifacies* and other mosquitoes in the ricefields, except in months (usually November each year) when there were almost daily rains, so that even 4 days' absence of irrigation water did not result in dry fields. The 5 wet : 1 dry cycle was not satisfactory as a control measure. Doubtless in cooler climates, and especially with more retentive soil, 4 days without water would not be long enough to effect drying of fields. Also, when fields are uneven so that pools remain in the dry period, some larvæ will survive.

Fortunately, in Pattukkottai, the malaria vector *A. culicifacies* (a) is not found in ricefields when the crop is more than a foot high and (b) declines markedly in seasonal density in October. Therefore, failure to control breeding in ricefields during the rains of November, when the rice crop is about half grown, is not a serious defect from the standpoint of malaria control.

But breeding of *A. culicifacies* in this area is always widespread and fairly intense in ricefields from the time the irrigation water comes on the fallow land in mid-June until about the end of September when, because of season and height of rice, the breeding of this species falls off. Therefore, we feel justified in asserting that the intermittent irrigation of ricefields according to the method described above would be of considerable value as an antimalaria measure in Pattukkottai.

The next point that must be considered is the effect of such a procedure on the rice crop, for any measure that reduces the yield of an agricultural crop cannot be classed as good practice in malaria control. We believe that this view is of great importance and that it must have careful consideration. Therefore, we carried out the experiments for three seasons to determine this point before forming an opinion, and we are continuing them on a larger scale for yet another season.

The net result of seven carefully controlled experiments on rented land, as described above, was to show that, except in the presence of fungus disease, intermittent irrigation as carried out did not significantly modify the growth of weeds or alter the yield of grain or straw. In the presence of fungus disease, it did not significantly modify growth of rice or weeds up to the flowering stage. From this time to the harvest it is possible, but by no means certain, that the intermittently irrigated plots were a little more susceptible to this disease. Since larvæ of *A. culicifacies* are uncommon in ricefields during the flowering stage, intermittent irrigation is not essential at that time for malaria control.



It is one thing to rent land and test intermittent irrigation under absolute experimental control, but another matter to try it under village conditions. The two experiments in villages, reported above, confirmed in general the results of the seven experiments on rented land. Experienced observers could see no significant differences between intermittently irrigated crops and those in contiguous areas which had had continuous irrigation. In one village (Alivalam) the ryots were pleased and requested that the experiment be continued. In the other village (Senjayakollai) the ryots complained of too much water, of too little water, and of any water at all! They also complained of mealy-bug infestation, which they themselves knew and stated to be no greater in their fields than elsewhere in the area. Impartial observers could not see any differences between Senjayakollai crops and those of other areas. But the complaints illustrated some of the difficulties of introducing any new element into the life of peasants. On the whole, however, the experiment was conducted without much trouble.

One important factor in each village was that we had control of the sluice gates, which we were able to lock open or closed, as required by the experiment. This control is essential. It is doubtful if in any part of South India the ryots themselves would, without supervision, carry out any organized community system of irrigation, although they are very ingenious in practising methods of irrigating their own fields.

### SUMMARY AND CONCLUSIONS.

The intermittent irrigation of rice, under the soil and climatic conditions prevailing in Pattukkottai Taluk, South India, is an effective measure for controlling mosquito breeding in fields and small channels, when cycles of 5 wet followed by 2, 3, or 4 dry days, respectively, are carried out. A 5 wet : 1 dry day cycle does not control breeding.

Dry periods not exceeding 4 days do not cause cracking or clod-formation in the soil of ricefields. The subsoil remains moist, although the surface film disappears.

During a period of daily rains, as in November when the north-east monsoon is active, it is not possible to dry the fields sufficiently to control mosquito breeding by withholding irrigation water even for four days. This is not an important defect, because the malaria vector, *A. culicifacies*, does not breed actively at that time.

In the absence of fungus disease, intermittent irrigation of rice throughout the entire growing season, with regular periods of from 1 to 4 dry days, does not lessen the yield of straw or grain, and does not stimulate the growth of weeds. It has no effect on the quality of the rice.

In the presence of fungus disease it is possible that intermittent irrigation during the flowering stage of rice may increase its susceptibility to this pest and cause a lessening of yield of grain, but not of straw. This point was by no means clear and requires further study.

Intermittent irrigation of rice in Pattukkottai Taluk can be recommended as a suitable antimalaria measure, if practised on an approximately 5 wet : 2 dry days' cycle from the time irrigation water arrives in mid-June, until the rice is in flower, by which time the vector, *A. culicifacies*, will have disappeared almost completely from ricefields, because of other factors, seasonal and mechanical.



## REFERENCES.

- ANANYAN, S. A. (1929) .. Interrupted irrigation of ricefields in Armenia. *Trop. Med. Vet.*, **7**, 1, pp. 47-55. (*Rev. Appl. Ent.*, B, **17**, pp. 117-118.)
- Idem* (1930) .. *Ibid.*, **8**, 8-9, pp. 39-47. (*Ibid.*, **19**, p. 154, 1931.)
- ENIKOLOPOV, S. K. (1931) .. Interrupted irrigation of ricefields. *Ibid.*, **9**, 2, pp. 58-63. (*Ibid.*, **20**, p. 114, 1932.)
- HILL, R. B., and CAMBOURNAC, F. J. C. (1941). Intermittent irrigation in rice cultivation, and its effect on yield, water consumption and Anopheles production. *Amer. J. Trop. Med.*, **21**, 1, pp. 123-144.
- KONSULOFF, S. (1922) .. The control of malaria mosquitoes in ricefields. *Z. Angew. Ent. Berlin*, **8**, 2, pp. 283-294. (*Rev. Appl. Ent.*, B, **10**, p. 193.)
- Idem* (1933) .. Irrigation and malaria. *Ibid.*, **20**, 3, pp. 461-463. (*Ibid.*, **22**, p. 52, 1934.)
- LEAGUE OF NATIONS (1937) .. French Indo-China, Rep. of Trop. Gen. Med. and P. H. Preparatory paper of Int.-Govt. Conf. Far-eastern countries on rural hygiene. Health Org. Document C.H. 1235.
- PROSOLUPOV, K. G. (1931) .. Observations on ricefields. *Trop. Med. Vet.*, **9**, 7-8, pp. 357-358. (*Rev. Appl. Ent.*, B, **20**, p. 116, 1932.)
- RUSSELL, P. F. (1938) .. Malaria due to defective and untidy irrigation. A preliminary discussion. *J. Mal. Inst. Ind.*, **1**, 4, pp. 339-349.
- RUSSELL, P. F., and EATON, L. S. (1934). An automatic distributing machine for paris green mixtures. *Phil. J. Sci.*, **53**, 4, pp. 497-503.
- RUSSELL, P. F., MENON, M. K., and RAO, T. R. (1938). Epidemiology of malaria in Pattukkottai Taluk, Tanjore District, Madras Presidency, India. *J. Mal. Inst. Ind.*, **1**, 3, pp. 285-326.
- RUSSELL, P. F., and RAO, H. R. (1940). The Anopheles of ricefields in South-eastern Madras. *Ibid.*, **3**, 4, pp. 427-446.
- RUSSELL, P. F., and RAO, T. R. (1940). Natural malaria infections in some South Indian anophelines, with special reference to *A. culicifacies*. *Ibid.*, **3**, 4, pp. 543-562.
- SMALT, F. H. (1937) .. The periodic draining of ricefields to control malaria. *Meded. Dienst. Volkgezondh. Ned.-Ind.*, **26**, 4, pp. 285-299. (*Rev. Appl. Ent.*, B, **26**, p. 105, 1938.)



## SOME NOTES ON THE PRACTICAL ASPECTS OF MOSQUITO CONTROL IN WELLS AND TANKS BY THE USE OF LARVIVOROUS FISH.

BY

R. BHASKER RAO, M.B., B.S., B.S.SC.  
(*Health Officer*),

AND

H. RAMOO, B.A.  
(*Entomological Assistant*).  
(*Antimalarial Scheme, Pattukkottai, Madras Presidency.*)

[December 22, 1941.]

### INTRODUCTION.

THE control of mosquito breeding by naturalistic methods has attracted considerable attention in recent years. Of these, the use of larvivorous fish has been recommended because there are objections to the application of larvicides to certain breeding places, such as domestic wells, ornamental waters and tanks. But there are a number of limitations and drawbacks to this method of control. Apart from the physiological increase or decrease observed at certain seasons of the year, the fish stocked in certain breeding places, particularly in wells, sometimes disappear almost completely after a while, from various causes, some of which are discussed below. Moreover, the control of mosquito breeding by fish is rarely so effective as that obtained by the use of larvicides. In this paper, attention is drawn to some of the practical aspects of the problem, and certain suggestions are put forward in order to obtain the best results. The data collected are from actual observations carried out at Pattukkottai, where an experiment in the control of rural malaria jointly financed by a special grant from the Government of India and by the Government of Madras has been in progress during the last three years.

There are approximately 50 tanks and 1,300 wells in Pattukkottai town, and in order to control mosquito breeding in them about 400 *Gambusia* fish were obtained from the Rockefeller Foundation Malaria Field Station. These were placed in certain canal borrowpits containing seepage water, pending the construction of a suitable hatchery. In the course of a few months they multiplied enormously, so that the numbers were sufficient to stock all the wells



in the town. Some of the wells were also stocked with *Panchax parvus*, a local species, which is found in many of the shallow tanks here. About 20 to 25 fish were introduced into each well, but two weeks later in most cases they had almost completely disappeared.

## CAUSES OF THE DISAPPEARANCE OF FISH IN WELLS.

### 1. ENEMIES.

(a) *Predatory fish*.—The first solution that suggested itself was the presence of other predatory fish. Enquiries revealed that it was a common practice here to keep a few fish locally known as *verrol* (belonging to the genus *Ophiocephalus*) in the wells in the belief that they would help to keep them clean by acting as scavengers, or possibly to afford food in times of need. It was, therefore, decided to destroy them. Various methods were suggested, such as the use of dynamite, fishing nets, the fixing of a wire-gauze screen about 6 inches below the level of the water in the wells and chlorination. The last-mentioned method was adopted in preference to all the others, which were not found feasible for various reasons. Percloron was used for chlorination. This has been reported upon favourably in Ceylon, and has also been tried locally with good results by Russell and Jacob (1939). About 5 to 6 ounces was found sufficient for about 800 gallons of water. After calculating the quantity of water in the well, the requisite amount of the substance is dissolved in a bucket of water. The bucket is lowered into the well and moved about in the water from side to side. Usually most of the medium-sized predatory fish are killed within an hour. It takes a few hours for the bigger ones to die. The dead fish, which float on the surface, can be removed by means of a net or some other contrivance, such as a basket tied to a long rope. Where bigger fish are found in large numbers, a slightly increased dose of percloron may be necessary. The suitability of the fish as food does not seem to be affected by this treatment, provided that they are washed thoroughly before being eaten. The larvivorous fish should be introduced only after all traces of chlorine have disappeared from the water. In wells which are in daily use, it takes a week or even longer for its complete disappearance. If desired, a test for dissolved chlorine in water may be made with starch-iodide paper.

(b) *Frogs* are also very commonly found in the wells and have been known to eat fish. Some frogs which had found their way into our *Gambusia* hatchery were dissected, and one of them was actually found to contain a fish inside its stomach (Plate XXXIV, fig. 1). After this discovery, the hatchery was provided with a wire-gauze cover. In deep wells where frogs are present in large numbers with no possible means of escape and where their supply of food is limited, the larvivorous fish introduced have been found to disappear within a few days. As it is very difficult to eliminate them from the types of wells found here, it is probable that frogs are one of the most formidable enemies of fish in such a confined area. Parapet walls, about 3 to 4 feet high, should prove effective in preventing frogs from entering the wells. The destruction of frogs in wells may be effected by fixing a wire screen, for a few hours, a few inches below the water surface, thus preventing them from coming to the surface to breathe. They will then be drowned.

(c) *King fishers*.—Where the subsoil water level is fairly high, king fishers have frequently been seen to eat the fish. Most of the local wells are devoid



PLATE XXXIV.



Fig. 1. A frog found in our Gambusia hatchery and dissected on 15.iv 40. showing a fish inside its stomach.

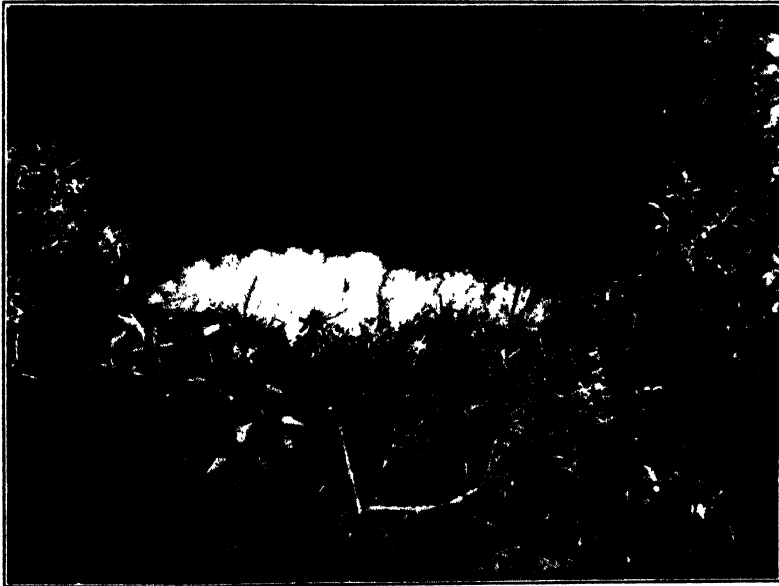


Fig. 2. A typical well in Pattukkottai







of parapet walls, and have no more protection than ordinary borrowpits (Plate XXXIV, fig. 2). Parapet walls may to some extent minimize the danger of exposing the fish to the view of birds.

(d) *Other enemies.*—The other common natural enemies which have been noticed in our observation wells and fish hatcheries are water snakes, water turtles, predaceous beetles and giant water-bugs. The last-named arthropod enemies should be removed as far as possible by the fish patrol staff during their rounds.

## 2. FOOD.

The next question to decide was whether the lack of food supply could have played any part in the disappearance of the fish. *Gambusia* are very catholic in their tastes. Although essentially carnivorous, they do not dislike vegetable and prepared foods. In our hatchery, they have often been fed on powdered cereals, such as wheat, for certain periods. An interesting observation recorded in our *Gambusia* hatchery was the habit of the young fish of chasing the bigger ones from behind and apparently attempting to feed on the proctodeal contents. As these fish are voracious feeders, it is possible that some undigested matter is passed out, which may serve as food for the younger ones. This is quite distinct from the chasing of the female by the male in courting, and from the pursuit of gravid females which are discharging their young in an attempt to devour them. Further, the usual food of these kind of fish is furnished by the aquatic stages of various insects, including mosquito larvæ. Wells stocked with larvicidal fish have been found to contain many kinds of these, even when the fish were steadily decreasing in numbers. This factor does not appear to be as important as was originally thought to be the case.

## 3. CHANGES IN DISSOLVED OXYGEN AND CARBON DIOXIDE.

Tests for dissolved oxygen were carried out at intervals of about a fortnight on the waters of certain wells stocked with larvicidal fish. No appreciable change was noticed which might account for the disappearance of the fish (Table).

## 4. OTHER FACTORS.

(a) *Diseases.*—*Tail-rot*, a disease due to the attack of a parasitic fungus, has been noticed in some of the fish introduced into certain wells and tanks. On one occasion, heavy mortality was also caused to the fish in our hatchery by this disease. When attacked with this disease, the fish are seen to float listlessly on the surface of the water. A small white patch appears at the side of the tail which gradually spreads and the infected fish soon die. The disease is highly infectious. The presence of filth and other putrefying matter in the water is said to be a predisposing cause of this disease. In the hatchery, treatment of the water with a little common salt or lime has been found effective in controlling it. It is advisable to remove the infected fish to prevent further spread of the disease.

In certain deep wells, some of the fish develop a bloated appearance, as though blown up with air. This may be due either to disease of the swim



TABLE.

*The oxygen and carbon-dioxide contents in water samples in Pattukkottai wells.*

Serial number.	1		2		3		4		5		6		7		8	
	O <sub>2</sub>	CO <sub>2</sub>	O <sub>2</sub>	CO <sub>2</sub>	O <sub>2</sub>	CO <sub>2</sub>	O <sub>2</sub>	CO <sub>2</sub>	O <sub>2</sub>	CO <sub>2</sub>	O <sub>2</sub>	CO <sub>2</sub>	O <sub>2</sub>	CO <sub>2</sub>	O <sub>2</sub>	CO <sub>2</sub>
1	3.1	1.4	5.9	1.3	12.6	1.2	13.3	0.5	4.9	0.5	..	..	..	..	..	..
2	6.3	1.1	5.0	1.3	4.4	1.1	6.0	0.7	3.5	0.9	3.3	1.3	2.2	2.0	4.5	1.0
3	4.4	2.0	1.9	2.1	1.1	2.2	2.4	1.9	1.9	1.4	..	..	5.1	1.0	2.0	3.0
4	2.1	1.1	3.0	2.1	1.8	1.7	2.2	1.0	2.0	0.9	2.3	1.9	1.8	1.7	4.3	1.3
5	3.9	1.6	2.6	2.0	1.6	1.6	1.2	1.2	2.0	1.2	2.8	1.6	2.7	1.4	3.1	1.6
6	3.0	1.8	3.2	1.8	..	2.3	1.9	1.0	2.2	1.5	2.7	1.4	2.6	1.8	3.8	1.6
7	3.7	3.4	2.3	3.3	2.0	2.6	..	..	3.9	0.7	2.2	1.2	3.7	1.3	3.1	1.3
8	3.7	2.1	3.4	2.6	1.5	1.6	2.6	0.9	3.3	1.1	2.2	1.6	3.0	1.6	2.8	2.1
9	1.1	2.8	2.5	2.4	1.5	2.0	1.8	1.5	3.5	2.1	2.8	1.6	4.3	2.4	3.5	2.5
10	1.5	1.6	1.6	1.4	1.3	1.3	1.5	0.5	2.4	1.2	2.4	0.6	..	..	..	..
11	4.1	3.7	4.4	2.9	2.6	3.8	2.6	3.1	3.6	3.1	..	..	2.9	3.0	3.3	3.1
12	3.5	3.2	1.7	1.8	1.4	1.4	2.9	2.0	3.0	1.8	2.3	1.8	2.9	2.0	3.0	1.8
13	5.1	2.2	3.3	1.8	4.5	2.0	4.4	1.5	4.5	1.3	3.7	1.9	6.6	1.6	..	..
14	2.8	2.0	3.7	4.4	3.4	2.1	2.0	0.9	1.9	1.7	3.0	1.4	2.7	1.7	1.2	1.8
15	2.7	2.6	3.1	2.7	3.3	2.2	1.5	1.8	1.9	2.3	2.5	1.8	3.1	1.4	3.6	2.7
16	2.8	3.1	2.4	2.6	2.9	3.3	1.4	2.4	1.0	2.5	2.6	2.3	3.1	2.0	2.6	1.5



17	44	33	19	30	08	29	14	17	20	16	12	23	30	25	20	19
18	17	35	21	34	21	34	15	25	24	25	32	22	25	24	15	26
19	20	20	17	25	19	34	20	16	25	17	20	30	30	26	16	30
20	24	31	20	28	07	37	27	22	25	26	23	49	29	38	28	28
21	09	32	07	23	16	27	16	11	15	22	09	25	07	25	11	23
22	14	16	16	18	23	18	23	08	24	12	34	15	21	16	16	14
23	19	27	21	33	29	29	46	15	16	23	24	32	35	26	21	21
24	44	40	21	25	28	23	14	24	26	22	32	20	28	25	53	23
25	29	40	25	24	20	30	22	20	37	18	31	15	34	20	34	16
26	26	34	25	31	33	41	24	16	50	21	34	18	36	21	40	22
27	40	20	28	20	17	44	26	15	38	14	42	08	38	14	24	20
28	32	46	27	28	23	32	25	30	36	26	37	16	22	27	37	24
29	17	42	18	23	30	25	22	20	23	21	24	19	31	25	34	20
30	10	24	18	23	20	17	20	16	28	14	19	12	23	15	22	17
31	27	48	14	29	28	19	28	22	24	18	08	15	18	22	19	18
32	36	36	22	29	18	36	31	16	40	14	24	18	35	21	49	19

Notes.—The figures give the quantity of oxygen in c.c. per litre of the sample and of carbon dioxide in parts per 50,000 of the sample.

The tests were carried out during the period commencing from 29.i.40 to 1.vii.40.



bladder or dropsy. Faulty diet is also said to be a cause of this condition. But there was no evidence to suggest that this caused any undue mortality, as many of the fish in such a condition were found to survive for a considerable time.

(b) *Accidental removal*.—We believe that a common cause for the depletion of the fish is their removal in the vessel in which the water is drawn up when the wells are in constant use. It has been observed that in certain wells in which fish flourish in spite of their being in daily use, numerous crevices are present in the walls. On the slightest disturbance of the water surface, the fish dart into one of these and so escape being caught in the vessel.

(c) *Disproportion of the sexes in the number of fish introduced*.—Absence of one or the other sexes among the fish with which the wells are stocked may result in their eventual disappearance. Care must, therefore, be taken that both sexes are present in the lot introduced. Mulligan and Majid (1936) suggest that in the case of *Gambusia* one or two males should be introduced for every 12 females. A few practical points for distinguishing the sexes of *Gambusia affinis* and *Panchax parvus* are given below :—

*Gambusia affinis*.—In the male the anal fin is modified into a straight rod-like projection called the *gonopodium*, which is curved backwards and lies parallel with the body on the under side. In very young males, only a few weeks old, the *gonopodia* are not fully developed, but the anal fin tends to be more pointed than that of the female.

*Panchax parvus*.—The males can be recognized by the presence of a number of scattered brownish spots on the tail, anal and dorsal fins. The anal fin of the male is larger than that of the female, and the males are more brightly coloured.

## CAUSES OF THE DISAPPEARANCE OF FISH IN TANKS.

In tanks, some of the adverse factors present in confined spaces, such as wells, do not seem to play such an important part. *Gambusia* generally do well, provided they can establish themselves before being destroyed by other predatory fish. *Panchax parvus*, which occurs naturally in some tanks, does not seem to need help and is able to take care of itself. Before stocking tanks with *Gambusia*, it has been our practice to drain them during the dry non-irrigation season and allow them to remain dry for a few days in order to destroy even the eggs of any predaceous fish. If any pool is left behind, it can easily be chlorinated and the predatory fish thus killed. After removing all the dead fish and allowing a few days for the effects of the chlorine to wear off, a few hundred *Gambusia* are introduced into the pool. Whenever it becomes necessary to fill the tanks, water from an irrigation canal is let in through a wire-gauze screen fixed to the inlet so as to prevent the entry of predatory fish. In the rainy season, there is a danger that the fish may be washed out, should the tanks overflow. During the first season, the outlet vent of the tank was also provided with a wire-gauze screen in order to prevent the fish escaping along with the discharged water. Where such precautions have been taken, *Gambusia* have thrived very well, excepting during the summer months, when they decline considerably in numbers. But in the absence of such measures, the *Gambusia* have failed to survive. It has been observed that they seem to prefer shallow sloping banks rather than those which are steep or vertical.



### SUMMARY.

(1) Some of the commoner causes for the disappearance of larvicidal fish stocked in wells and tanks are described, and measures for obtaining the best results from this method of mosquito control are suggested.

(2) The necessity for periodical inspection of breeding places and of re-stocking them where necessary in a mosquito control campaign is stressed. For this purpose, it is essential to maintain a suitable hatchery.

### ACKNOWLEDGMENTS.

We wish to thank Dr. Paul F. Russell and Mr. Fred. W. Knipe of the Rockefeller Foundation Malaria Research Unit for their constant help and advice, Dr. S. G. Masillamani, Malaria Officer, Madras, for useful suggestions, and Dr. R. Adiseshan, Director of Public Health, Madras, for permission to publish this article.

### REFERENCES.

- MULLIGAN, H. W., and MAJID, ABDUL. Some notes on the care, transportation, and use of *Gambusia affinis* under Indian conditions. *Rec. Mal. Surv. Ind.*, **6**, pp. 537-547.
- RUSSELL, P. F., and JACOB, V. P. Some experiments in the use of fish to control *Anopheles* breeding in casuarina-pits. *J. Mal. Inst. Ind.*, **2**, pp. 273-291.







## ON THE EPIDEMIOLOGY OF MALARIA IN THE NILGIRIS DISTRICT, MADRAS PRESIDENCY\*.

BY

PAUL F. RUSSELL

AND

V. P. JACOB.

[January 6, 1942.]

### INTRODUCTION.

THIS paper describes the epidemiology of malaria in the Nilgiris area of Madras Presidency, in south-western India, on the basis of data collected from January 1940 to January 1941, inclusive.

The Nilgiris ('Blue Mountains') area includes: (1) a central plateau some 35 miles long and 20 miles wide, averaging about 6,500 feet in altitude and including the towns of Ootacamund (7,500 feet) and Coonoor (6,000 feet); (2) a steep jungly valley to the east, dropping through Kallar to the Mettupalaiyam plains, which have an altitude of about 1,000 feet; (3) a steep descent to the west, dropping to the Nilgiris portion of the Wynaad ('land of swamps'), a tableland of some 3,000 feet elevation, consisting of bamboo forest, paddy-flats, small streams, and bogs, merging into the Malabar-Wynaad further west; (4) a steep cliff descending to the Bhavani river, a natural boundary on the south; (5) another cliff-like boundary to the north, descending to the Mysore plateau, which has an altitude of about 2,500 feet.

Politically, the districts bounding the Nilgiris are as follows: north-east, east, and south-east—Coimbatore; south-west, west, and north-west—Malabar. The north central portion borders on Mysore State (Map). The area of

---

\* These studies were made under the auspices, and with the support, of the International Health Division of the Rockefeller Foundation, co-operating with the Health Department of Madras Presidency and the Pasteur Institute of Southern India.



Malabar contiguous to and north-west of the Nilgiris is known as the Wynaad, which is similar in most respects to what we have referred to as Nilgiris west. Covell and Harbhagwan (1939) have reported on the malaria of that area, and there it appears to have much the same epidemiology as in Nilgiris west.

In the following discussion and tables we have arbitrarily called the central plateau, Nilgiris central. We have considered the eastern valley, including Kallar (actually in Coimbatore District), as Nilgiris east. The Nilgiris-Wynaad

MAP OF NILGIRIS AREA.

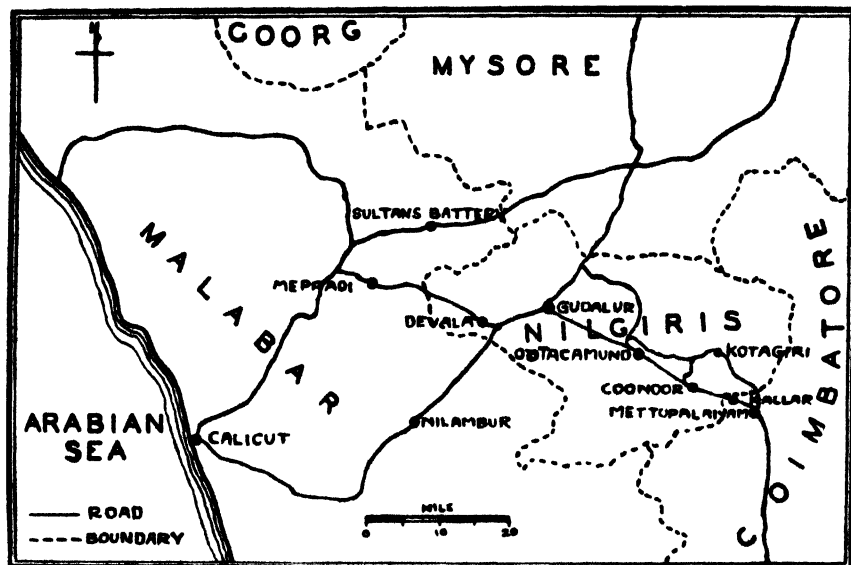


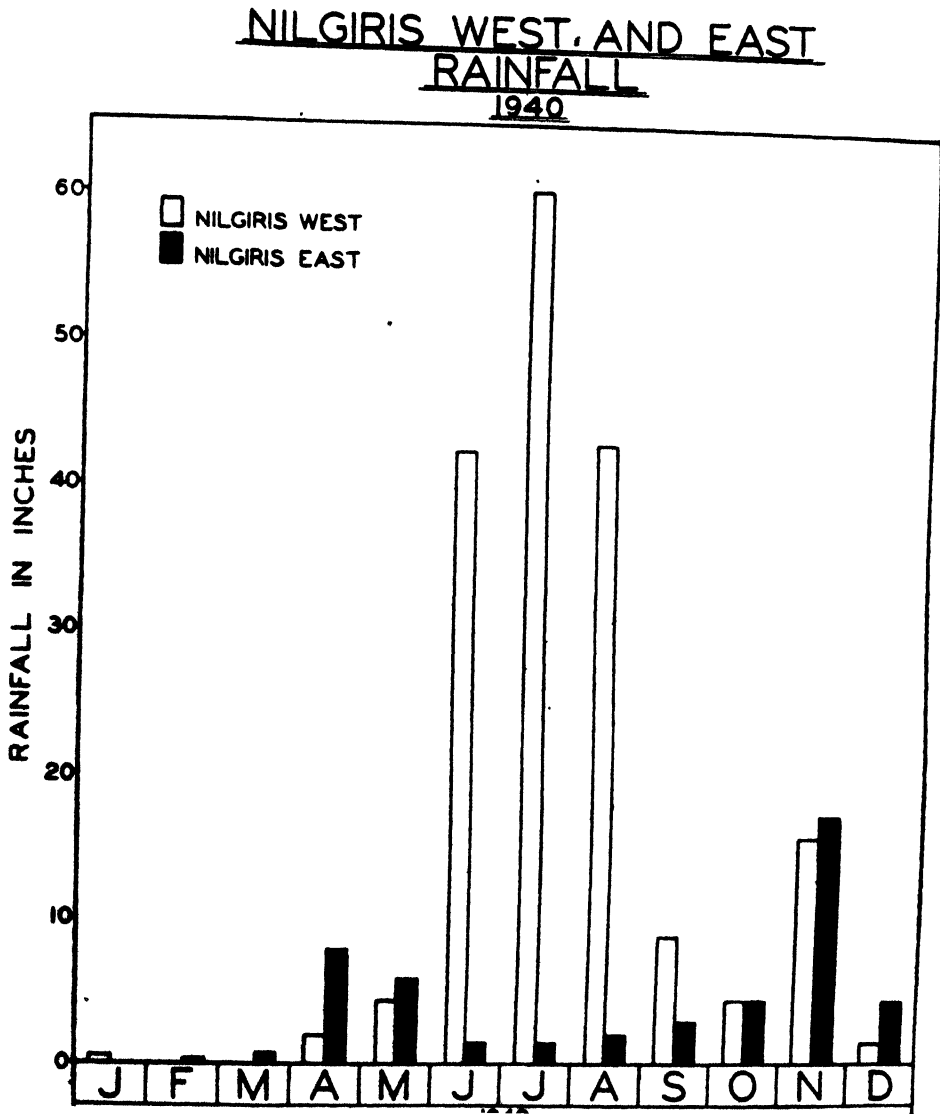
table-land, which includes Gudalur and Devala, we have called Nilgiris west. The steep uninhabited cliffs to the north and south have not been included in this survey.

The three arbitrary divisions we have made show decided differences as regards rainfall, altitude, and the seasonal distribution of malaria and its vector, *A. fluviatilis*. Nilgiris east has a rainfall averaging about 55 inches, fairly well distributed, although usually greater in October and/or November when the north-east monsoon occurs (Chart 1). The streams at Kallar were not well flushed out in 1940, but in some years there have been heavy spates. The altitude of Kallar is about 1,500 feet. Nilgiris central, as typified by Coonoor, has a rainfall averaging about 65 inches, fairly well distributed, generally highest in October and/or November. Nilgiris west, on the other hand, as typified by Gudalur, has an annual rainfall averaging over 150 inches, more than half falling in the months of June, July and August, during the south-west monsoon. In 1940, more than 60 inches of a total of 186 inches fell in July, and over 40 inches each in June and August (Chart 1). All streams, field and irrigation channels, and ditches were thoroughly flushed out, so that the effect was marked on a species such as *A. fluviatilis*, which breeds in running water.



In all parts of the Nilgiris, temperature and relative humidity are usually compatible with mosquito activity. Average minimum night temperatures in

CHART 1.  
Rainfall in inches. Nilgiris east and west, by months, 1940.



Coonoor in December, January, or February may go a little below 50°F., but average maximum day temperatures during those months are over 60°F. (Tables I to III).



There are numerous small streams throughout the area, and in both Kallar and Gudalur there are irrigation and field channels for rice, plantain, and areca-

TABLE I.  
*Meteorology, Nilgiris west.*

Month.	1937.	1938.						1939.	1940.
	Rainfall (inches).	Rainfall (inches).	Tempera- ture (°F.).		Relative humidity, per cent.		Rainfall (inches).	Rainfall (inches).	
			Maxi- mum.	Mini- mum.	8 a.m.	4 p.m.			
January ..	0.00	0.00	83.0*	58.4*	82.6	42.0	0.07	0.36	
February ..	0.29	0.18	87.5*	60.1*	77.2	63.5	0.00	0.00	
March ..	1.52	0.22	89.1	66.6	87.5	66.4	1.73	0.00	
April ..	5.27	3.63	89.5	67.6	90.1	72.7	3.61	1.97	
May ..	5.21	9.25	87.8	66.7	87.2	70.1	2.23	4.56	
June ..	24.98	36.74	76.6	64.5	90.6	86.9	27.82	42.46	
July ..	47.75	25.35	75.0	63.9	91.2	88.2	58.37	60.72	
August ..	19.33	23.86	78.6	65.1	91.3	88.2	45.02	43.02	
September ..	9.44	18.96	80.1	66.5	89.0	80.6	9.04	10.97	
October ..	29.37	14.97	79.5	64.5	88.9	79.5	22.04	4.33	
November ..	6.66	4.17	80.5	62.2	87.2	64.5	5.69	16.12	
December ..	0.61	0.54	80.9	59.7	85.9	60.7	0.00	1.53	
TOTAL ..	144.43	137.87	..	..	..	..	175.62	186.04	
AVERAGE ..	..	..	82.3	63.8	87.4	71.9	..	..	

*Note.*—Rainfall figures by courtesy of Rowsden Mulla Tea Estate, Devala. Other figures from Covell and Harbhagwan (1939).

\* Figures for 1939.

nut culture. At higher elevations, a considerable amount of tea and coffee are grown and there is a cinchona plantation between Gudalur and Ootacamund.



TABLE II.  
*Meteorology, Nilgiris east.*

Month.	1937.				1938.				1939.				1940.			
	Rainfall (inches).	Mean temperature. (°F.)		Rainfall (inches).	Mean temperature. (°F.)		Rainfall (inches).	Mean temperature. (°F.)		Rainfall (inches).	Mean temperature. (°F.)		Rainfall (inches).	Mean temperature. (°F.)		
		Maxi- mum.	Mini- mum.		Maxi- mum.	Mini- mum.		Maxi- mum.	Mini- mum.		Maxi- mum.	Mini- mum.				
January ..	0.67	86.1	63.1	0.00	85.3	63.3	2.30	84.5	63.2	0.00	81.3	60.4				
February ..	19.36	88.5	65.2	13.08	88.5	65.1	0.00	87.6	66.4	0.14	89.1	66.2				
March ..	3.95	98.0	68.9	1.73	94.7	66.3	2.32	92.0	69.7	0.74	94.5	68.7				
April ..	5.67	96.9	68.0	5.09	89.8	66.4	4.93	92.0	69.8	7.83	93.9	68.7				
May ..	2.14	94.9	69.1	1.90	93.7	68.1	2.96	95.7	68.1	6.24	88.3	67.9				
June ..	3.29	92.3	65.9	0.16	90.2	66.9	1.66	91.3	72.0	1.34	88.8	67.9				
July ..	3.02	84.5	63.6	2.68	87.7	65.2	0.45	90.8	68.5	1.37	86.7	69.0				
August ..	2.97	90.2	69.3	4.83	88.1	67.8	3.93	90.2	69.8	1.85	86.7	67.0				
September ..	1.73	93.5	69.9	2.98	87.0	67.1	3.13	89.2	68.6	3.02	90.3	67.7				
October ..	16.04	87.8	65.9	4.72	89.2	67.3	14.62	86.7	65.2	4.29	88.1	68.4				
November ..	11.88	83.7	62.6	4.70	86.4	64.7	7.51	84.1	64.3	17.34	84.3	66.6				
December ..	2.42	86.7	63.9	4.00	84.4	63.1	8.12	82.4	62.4	4.34	84.0	64.3				
TOTAL ..	73.14	..	..	45.87	..	..	51.93	..	..	48.50	..	..				
AVERAGE ..	..	90.3	66.3	..	88.8	65.9	..	88.9	66.5	..	88.0	66.9				

Note.—Figures by courtesy of the Government Fruit Gardens at Kallar.



**TABLE III.**  
*Meteorology, Nilgiris central, Coonoor.*

Month.	1937.				1938.				1939.				1940.			
	Rainfall (inches).	Mean temperature. (°F.)		Rela- tive humidi- ty mean.	Rainfall (inches).	Mean temperature. (°F.)		Rela- tive humidi- ty mean.	Rainfall (inches).	Mean temperature. (°F.)		Rela- tive humidi- ty mean.	Rainfall (inches).	Mean temperature. (°F.)		Rela- tive humidi- ty mean.
		Maxi- mum.	Mini- mum.			Maxi- mum.	Mini- mum.			Maxi- mum.	Mini- mum.			Maxi- mum.	Mini- mum.	
January ..	1.05	67.4	46.8	66.9	0.00	67.5	46.9	66.8	4.11	61.4	47.0	59.0	0.06	64.5	45.5	75.0
February ..	6.79	70.0	52.2	78.4	7.56	69.0	52.6	73.8	0.71	57.9	46.7	64.8	1.34	70.0	48.4	51.0
March ..	6.33	72.5	53.7	68.3	9.41	73.8	53.0	58.4	1.55	67.9	51.2	49.0	0.38	74.3	52.8	53.0
April ..	8.12	73.1	56.9	66.9	6.80	73.8	57.0	68.4	9.69	73.1	55.8	64.7	9.67	74.7	55.8	64.0
May ..	2.76	76.8	59.3	58.7	1.41	76.8	59.1	59.5	3.32	77.7	58.8	52.7	7.13	75.5	58.9	60.0
June ..	3.58	73.1	60.2	69.2	1.18	71.6	60.1	69.5	4.57	72.6	59.7	65.0	5.26	72.1	59.3	69.0
July ..	2.89	70.1	59.4	73.8	3.48	70.7	59.3	69.5	1.60	70.4	58.6	68.0	0.97	71.1	59.3	73.0
August ..	2.35	71.1	57.1	66.3	5.42	70.7	57.1	73.3	2.45	71.4	57.8	67.6	4.69	71.4	58.3	70.0
September ..	2.51	71.3	56.0	68.2	5.55	70.4	56.9	72.7	5.41	71.3	55.8	64.3	3.91	70.9	56.0	72.0
October ..	11.98	68.3	56.4	79.1	9.86	70.8	55.3	68.0	10.23	70.9	57.1	74.0	8.34	69.7	54.9	78.0
November ..	10.12	63.6	53.2	74.9	2.71	67.3	50.5	71.4	9.51	67.0	53.6	78.2	20.34	65.9	54.9	85.0
December ..	2.94	65.4	48.0	71.9	12.42	65.3	50.1	65.0	1.82	65.7	48.1	71.5	2.45	66.5	49.5	78.4
Total ..	61.42	..	..	..	65.80	..	..	..	54.97	..	..	..	64.54	..	..	..
Average ..	..	70.2	54.9	70.2	..	70.6	54.8	68.0	..	68.9	54.2	64.9	..	70.5	54.4	69.0

*Note.*—Figures by courtesy of Municipal Office, Coonoor.



## ANOPHELINES.

The anopheline fauna of the Nilgiris is extraordinarily rich, and we found 23 species in our collections during 1940, as shown in the following list:—

*Anophelines collected in Nilgiris, 1940.*

1. *A. acronitus* Dönitz, 1902.
2. *A. aitkeni* James, 1903.
3. *A. annandalei* var. *interruptus* Puri, 1929.
4. *A. annularis* van der Wulp, 1884.
5. *A. barbirostris* van der Wulp, 1884.
6. *A. culicifacies* Giles, 1901.
7. *A. fluviatilis* James, 1902.
8. *A. gigas* Giles, 1901.
9. *A. gigas* var. *simlensis* James, 1911.
10. *A. hyrcanus* var. *nigerrimus* Giles, 1900.
11. *A. jamesi* Theobald, 1901.
12. *A. jeyporiensis* James, 1902.
13. *A. karwari* James, 1903.
14. *A. leucosphyrus* Dönitz, 1901.
15. *A. lindesayi* var. *nilgircus* Christophers, 1924.
16. *A. maculatus* Theobald, 1901.
17. *A. majidi* McCombie Young and Majid, 1928.
18. *A. pallidus* Theobald, 1901.
19. *A. splendidus* Koidzumi, 1920.
20. *A. subpictus* Grassi, 1899.
21. *A. tessellatus* Theobald, 1901.
22. *A. vagus* Dönitz, 1902.
23. *A. varuna* Iyengar, 1924.

Covell and Puri (1936) listed 29 species that had been recorded from Madras Presidency up to that time. We can now add two other species, viz., *A. annandalei* var. *interruptus* and *A. gigas* var. *simlensis*, making a total (with *insulæflorum* mentioned below) of 32 species from Madras. Of this total we failed to collect the following nine species:—

- A. culiciformis.*
- A. insulæflorum.*
- A. minimus.*
- A. moghulensis.*
- A. philippinensis.*
- A. sintoni.*
- A. stephensi.*
- A. theobaldi.*
- A. turkhudi.*

Covell and Harbhagwan (*loc. cit.*) in the Wynaad, north-west of the Nilgiris, collected 20 species, including *A. insulæflorum*, not mentioned by Covell and Puri (*loc. cit.*). The identification of our specimens of *A. annandalei* var. *interruptus* was confirmed by courtesy of Dr I. M. Puri.



*Anopheles* larvae, all species, by totals and type of breeding place, collected in Nilgiris west, February 1940 to January 1941.

**\*Found in cut bamboo and plantain stalks.**



**TABLE V.**  
*Anopheles* larvae, all species, by totals and type of breeding place, collected in  
Nilgiris east, February 1940 to January 1941.

Type of breeding places.	<i>A. aconitus.</i>	<i>A. aikeni</i> type.	<i>A. annandalei</i> var. <i>interruptus.</i>	<i>A. annularis.</i>	<i>A. barbivostri.</i>	<i>A. culicifacies.</i>	<i>A. fluviatilis.</i>	<i>A. gigas</i> type.	<i>A. gigas</i> var. <i>simlensis.</i>	<i>A. hyrcanus</i> var. <i>nigerrimus.</i>	<i>A. jamesti.</i>	<i>A. jeyporicensis.</i>	<i>A. karwar.</i>	<i>A. leucosphyrus.</i>	<i>A. lindesayi</i> var. <i>nilgircus.</i>	<i>A. maculatus.</i>	<i>A. mayidi.</i>	<i>A. pallidus.</i>	<i>A. splendidus.</i>	<i>A. subpictus.</i>	<i>A. tessellatus.</i>	<i>A. vagus.</i>	<i>A. varuna.</i>	Total.
Tree-hole ..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..
Artificial container	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	3	..	..	..	1	..	11	..	15
Pit ..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..
Cart-track, hoof-mark.	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..
Ditch ..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	2	..	..	..	1	..	..	..	3
Ricefield fallow ..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..
Ricefield growing	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..
Ricefield channel	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..
Swamp ..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..
Hill-stream ..	..	239	..	..	9	61	440	3	11	6	1	54	24	84	1	832	..	..	5	4	..	13	..	1,787
River edge ..	..	..	..	..	..	192	7	..	..	..	..	..	..	..	..	2	..	..	..	125	63	..	..	389
River pool ..	..	..	..	..	..	152	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	152
Rainwater pool ..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..
Spring pool ..	..	..	..	..	..	..	..	..	..	..	20	8	..	..	..	12	..	..	..	..	..	..	..	40
Irrigation channel	..	43	..	..	3	..	64	..	..	1	1	9	..	..	..	97	..	..	1	2	1	..	..	222
Tank or pond ..	1	..	..	..	90	..	..	..	..	14	8	2	..	..	..	19	..	..	..	26	3	7	..	170
Well ..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..
TOTAL ..	1	282	..	..	102	405	511	3	11	21	30	73	24	84	1	967	..	..	6	158	5	94	..	2,778



TABLE VI.

*Anopheles* larvae, all species, by totals and type of breeding place, collected in Nilgiris central (Coonoor), February 1940 to January 1941.

Type of breeding places.	<i>A. aconitus</i> .	<i>A. aikeni</i> type.	<i>A. annandalei</i> var. <i>interruptus</i> .	<i>A. annularis</i> .	<i>A. barbivittatus</i> .	<i>A. culicifacies</i> .	<i>A. fluviatilis</i> .	<i>A. gignis</i> var. <i>sinhalensis</i> .	<i>A. hyrcanus</i> var. <i>nigerinus</i> .	<i>A. jamesti</i> .	<i>A. jeyporiensis</i> .	<i>A. karwari</i> .	<i>A. leucosphyrus</i> .	<i>A. lindesayi</i> var. <i>nigriticus</i> .	<i>A. maculatus</i> .	<i>A. majidi</i> .	<i>A. pallidus</i> .	<i>A. splendens</i> .	<i>A. subpictus</i> .	<i>A. tessellatus</i> .	<i>A. vagus</i> .	<i>A. varuna</i> .	Total.
Tree-hole ..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..
Artificial container ..	..	1	..	..	8	..	..	5	..	..	..	..	10	..	..	..	..	..	..	..	..	..	24
Pit ..	..	..	..	..	..	..	..	..	..	..	..	..	..	1	3	..	..	..	..	..	..	..	5
Cart-track, hoof-mark.	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..
Ditch ..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	12	..	..	..	..	..	..	..	12
Ricefield fallow ..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..
Ricefield growing ..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..
Ricefield channel ..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..
Swamp ..	..	229	..	..	..	..	..	11	..	..	2	..	..	..	6	..	..	..	..	..	..	..	249
Hill-stream ..	..	1,111	..	10	201	15	..	234	21	..	10	..	..	115	792	..	..	4	2	..	25	..	2,589
River edge ..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..
River pool ..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..
River bed pool ..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..
Rainwater pool ..	..	1	..	..	..	..	..	83	..	..	9	..	..	..	..	..	..	..	..	..	..	..	95
Spring pool ..	..	143	..	4	..	..	..	113	..	..	86	..	..	..	41	..	..	1	..	..	..	..	530
Irrigation channel ..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..
Tank or pond ..	..	5	..	..	..	..	..	..	..	..	1	..	..	..	..	..	..	..	..	..	..	..	28
Well ..	..	5	..	..	..	1	..	12	..	..	..	..	..	..	46	..	..	..	..	..	..	..	64
Total ..	..	1,495	..	14	209	16	..	444	231	..	108	..	10	116	900	..	..	5	2	..	25	..	3,596



The types of breeding place in which various species were collected were as follows (Tables IV, V and VI, and Text-figs. 1 and 2).

## Text-figure 1.

Card used for recording and analysing larval collections (obverse).

No.	Species	Larvae identified.	Adults hatched out		
			M	F	T
20	<i>Aconitus</i>				
21	<i>Aitkeni</i>				
22	<i>Annularis</i>				
23	<i>Barbinostris</i>				
24	<i>Culicifacies</i>				
25	<i>Fluvialis</i>				
26	<i>Gigas</i> (type)				
27	<i>Gigas v. similis</i>				
28	<i>Hyrcaus v. nigerrimus</i>				
29	<i>Insulæflorum</i>				
30	<i>Jamesi</i>				
31	<i>Jeyporiensis</i>				
32	<i>Karwari</i>				
33	<i>Leucosphyrus</i>				
34	<i>Lindesayi v. nilgiris</i>				
35	<i>Maculatus</i>				
36	<i>Majidi</i>				
37	<i>Pallidus</i>				
38	<i>Splendidus</i>				
39	<i>Stephensi</i>				
40	<i>Subpictus</i>				
41	<i>Tessellatus</i>				
42	<i>Vagus</i>				
43	<i>Varuna</i>				
44					
45					
Total identified					
Total caught	Stages 1 & 2	Stages 3 & 4	Pupae	Total	

REMARKS.

*A. aconitus*: hill-streams mostly, but also in tanks.

*A. aitkeni*: ricefields (fallow and growing), field and irrigation channels, swamps, hill-streams, rainwater pools, seepage pools and wells. Largest numbers in hill-streams.

*A. annandalei* var. *interruptus*: cut plantain stocks and cut bamboo stems.

*A. annularis*: ricefields (fallow and growing), swamps, hill-streams, spring pools, tanks. Largest numbers in spring pools.



Text-figure 2.

Card used for recording and analysing larval collections (reverse).

GENERAL CHARACTER OF BREEDING PLACE																	
Number.	Date	Malaria Investigations Collections of Larvae										PASTEUR INSTITUTE, COONOR, NILGIRIS FIELD AREA.					
Breeding Place																	
Temp. Water				Area Dipped				Time Spent Dipping									
REMARKS.																	
Number of dips																	
1	Tree hole	22	Annularis	43	Varuna												
2	Artificial container	23	Barbirostris	44													
3	It	24	Culicifacies	45													
4	Cart-track, Hoofmark	25	Fluviatilis	46	Stagnant												
5	Ditch	26	Gigas (type)	47	Dense shade												
6	Rice field fallow	27	Gigas v. similensis	48	Temporary												
7	Rice field growing	28	Hyrceanus v. nigerrimus	49	Very turbid												
8	Rice field channel	29	Insulaeflorum	50	Green algae												
9	Swamp	30	Jamesi	51	Blue Green algae												
10	Hill stream (above 1000 ft)	31	Jeyporiensis	52	No vegetation												
11	River edge	32	Kurwar	53	Emergent vegetation												
12	River pool	33	Leucosphyrus	54	Floating vegetation												
13	Rain water pool	34	Lindeasy v. nilgiris	55	Earth bottom												
14	Spring pool	35	Maculatus	56	Rock bottom												
15	Irrigation channel	36	Majidi	57	Sand bottom												
16	Tank or pond	37	Pellidus	58	Decaying vegetation												
17	Well	38	Splendidus	59													
18		39	Stephensi	60													
19		40	Subpictus	61	No larvae found												
20	Aconitus	41	Tessellatus	62	Collector												
21	Aitkeni	42	Vagus														
PARTICULARS																	
SPECIES																	

*A. barbirostris*: artificial containers, ricefields (fallow and growing), field and irrigation channels, hill-streams, spring pools, tanks and wells. Largest numbers in hill-streams, tanks and wells.

*A. culicifacies*: fallow ricefields, field channels, hill-streams, river edges and pools, spring pools and wells. Largest numbers along river edges and in river pools.

*A. fluviatilis*: ricefields (fallow and growing), field and irrigation channels, hill-streams, river edges, spring pools and wells. Largest numbers in field channels and hill-streams, with generally moderate to no shade, but sometimes fairly dense natural cover (Plate XXXV, figs. 1, 2; Plate XXXVI, fig. 3).

*A. gigas*: borrowpits, field channels, swamps, hill-streams, rainwater and spring pools and wells. Largest numbers in hill-streams and spring pools.

*A. gigas* var. *simlensis*: artificial containers, fallow ricefields, swamps, hill-streams, rainwater and spring pools, tanks and wells. Largest numbers in hill-streams and spring pools.

*A. hyrcanus* var. *nigerrimus*: ricefields (fallow and growing), field and irrigation channels, hill-streams, spring pools, tanks and wells. Largest numbers in hill-streams and wells.

*A. jamesi*: growing ricefields, field and irrigation channels, swamps, hill-streams, rainwater and spring pools, tanks and wells. Largest numbers in tanks and spring pools.

*A. jeyporiensis*: ricefields (fallow and growing), field and irrigation channels, swamps, hill-streams, rainwater and spring pools, tanks and wells. Largest numbers in ricefields, hill-streams, spring pools and tanks.



PLATE XXXV.



Fig. 1. Typical small stream at Kallar, Nilgiris east.  
A breeding place of *A. fluviatilis*.



Fig. 2. Typical ricefield surrounded by hills, at Gudalur, Nilgiris west. Small streams and channels are breeding places of *A. fluviatilis*.



PLATE XXXVI.



Fig. 3 Typical small stream at Gudalur, Nilgiris west. A breeding place of *A. fluviatilis*.



*A. karwari*: field channels, swamps, hill-streams, tanks and wells.

*A. leucosphyrus*: artificial containers, hill-streams and wells. Largest numbers in hill-streams.

*A. lindesayi* var. *nilgircus*: usually in hill-streams. Once found in a borrowpit.

*A. maculatus*: artificial containers, borrowpits, cart-tracks, ditches, fallow and growing ricefields, field and irrigation channels, swamps, hill-streams, river edges, spring pools, tanks and wells. Largest numbers in hill-streams, fallow ricefields, tanks and wells.

*A. majidi*: ricefields (fallow and growing), field channels, hill-streams, tanks and wells. Largest numbers in channels.

*A. pallidus*: collected once in growing ricefields.

*A. splendidus*: growing ricefields, field and irrigation channels, hill-streams, and spring pools.

*A. subpictus*: artificial containers, growing ricefields, hill-streams, river edges, irrigation channels and tanks. Largest numbers at river edges.

*A. tessellatus*: ditches, irrigation channels and tanks.

*A. vagus*: artificial containers, growing ricefields, field channels, hill-streams, river edges and tanks. Largest numbers along river edges.

*A. varuna*: hill-streams and tanks.

Seasonal distribution of larvæ is indicated in Tables VII, VIII and IX. The first two tables include all species collected, by months. In Table IX, collections of *A. fluviatilis* in Nilgiris west are contrasted with those in Nilgiris east, by weekly records. As one would expect from the marked difference in amount and distribution of rainfall in the two areas, east and west, there were marked seasonal differences in prevalence of larvæ. These were most marked in the case of *A. fluviatilis*, the vector (*see* below). In the west, where heavy south-west monsoon rains were experienced from July to September, this running-water species almost disappeared during those months. In the east at Kallar, where rainfall was much more evenly distributed, this species was common throughout the year (Charts 2 and 3).

As shown in Table X, altitude appeared to be a factor in determining the distribution of Anopheles species. *A. aitkeni*, for example, was most often taken at altitudes of 4,000 feet or more. *A. fluviatilis* was only found in our survey below 4,000 feet. *A. culicifacies* was most common below 2,000 feet. A few *A. gigas* were found below 4,000 feet but most were taken at higher altitudes. *A. subpictus* was not very abundant and was taken mostly below 2,000 feet.

In this connection, it may be recalled that Holt and Russell (1932), in an extensive survey in the Philippine Islands, failed to find the vector (*A. minimus* group) at higher altitudes than 2,000 feet. They noted that altitudes of less than 2,000 feet, *per se*, had no marked effect on the prevalence of malaria. It was apparently *change of contour* that directly affected malaria prevalence, for the *A. minimus* group bred chiefly in streams, rivers, and irrigation ditches in the foothills. Much the same can be said about malaria and *A. fluviatilis* in the Nilgiris, if one puts the upper limit at about 4,000 rather than 2,000 feet.

This elevation of 4,000 feet must not be taken as absolute, for there seems good reason to believe that in some seasons (especially with unusually



TABLE VII.  
*Anopheles larvæ, by species and months (larvæ per 15 minutes collecting), Nilgiris west, February 1940 to January 1941.*

Month and year.	<i>A. aconitus.</i>	<i>A. aikeni</i> type.	<i>A. annandalei</i> var. <i>interruptus.</i>	<i>A. annularis.</i>	<i>A. barbrosus.</i>	<i>A. culicifacies.</i>	<i>A. fluviatilis.</i>	<i>A. gyes</i> type.	<i>A. gyes</i> var. <i>sinlensis.</i>	<i>A. hyrcanus</i> var. <i>nigerinus.</i>	<i>A. juneisi.</i>	<i>A. jayporensis.</i>	<i>A. karwari.</i>	<i>A. leucosphyrus.</i>	<i>A. lindesayi</i> var. <i>nilgircus.</i>	<i>A. maculatus.</i>	<i>A. maydi.</i>	<i>A. pallidus.</i>	<i>A. splendens.</i>	<i>A. subpictus.</i>	<i>A. tessellatus.</i>	<i>A. vagus.</i>	<i>A. varuna.</i>
February 1940	..	8.6	..	..	3.4	..	11.0	..	..	4.0	2.0	6.3	..	..	..	7.6	1.0	..	..	..	..	..	..
March "	..	1.2	..	4.0	10.3	1.0	3.0	..	12.1	..	2.5	3.8	..	..	..	5.8	..	4.0	..	1.0	..	2.7	..
April "	..	3.0	..	7.8	4.1	1.0	4.7	..	1.0	4.8	2.0	5.3	..	..	..	6.1	..	1.0	..	..	..	2.5	..
May "	..	5.8	..	2.5	6.2	2.3	7.4	..	..	8.3	2.7	7.2	..	8.0	..	11.4	1.0	..	1.0	6.5	..	6.0	..
June "	12.0	6.0	..	6.0	4.9	..	9.5	..	7.0	3.0	3.3	9.3	..	..	..	6.5	..	..	3.0	1.0	..	10.5	2.8
July "	..	4.7	..	..	4.3	..	..	..	..	..	2.0	3.0	..	1.5	..	2.6	..	..	..	..	..	..	..
August "	..	1.5	..	..	6.0	..	..	..	2.0	..	1.5	10.0	..	..	..	3.4	..	..	..	..	..	1.0	..
September "	..	2.0	..	5.0	9.3	..	1.0	..	..	1.5	12.2	14.3	1.0	1.0	..	6.3	..	..	..	..	..	9.0	..
October "	..	2.4	..	..	3.3	3.0	3.0	3.3	..	1.3	3.3	2.9	5.5	..	..	2.0	4.0	..	2.0	..	..	..	..
November "	..	2.9	..	3.3	5.7	4.0	..	..	..	3.0	5.3	7.5	2.8	..	..	3.0	2.5	..	..	..	..	6.0	4.0
December "	..	..	..	2.0	13.5	..	..	..	..	15.0	9.0	7.5	1.0	..	..	4.8	3.0	..	..	..	..	..	..
January 1941	..	1.0	4.0	6.0	2.0	3.7	1.0	..	..	3.2	8.0	9.3	..	..	1.0	1.5	10.0	..	..	1.0	..	..	1.0
Average per 15 minutes.	12.0	3.6	4.0	4.6	6.1	2.5	5.1	3.3	3.3	5.6	4.5	7.2	2.6	3.5	1.0	5.1	3.6	2.5	2.0	2.4	..	5.4	2.6



TABLE VIII.  
*Anopheles* larvæ, by species and months (larvæ per 15 minutes collecting), Nilgiris east, February 1940  
to January 1941.

Month and year.	<i>A. acontus</i> .	<i>A. aikeni</i> type.	<i>A. annandalei</i> var. <i>interruptus</i> .	<i>A. annulus</i> .	<i>A. barbrosalis</i> .	<i>A. culicifacies</i> .	<i>A. fluviatilis</i> .	<i>A. gignis</i> type.	<i>A. gignis</i> var. <i>simlensis</i> .	<i>A. hyrcanus</i> var. <i>nigritimus</i> .	<i>A. jamesti</i> .	<i>A. jayporensis</i> .	<i>A. karwar</i> .	<i>A. leucosphyrus</i> .	<i>A. lindesayi</i> var. <i>nilgircus</i> .	<i>A. maculatus</i> .	<i>A. maidi</i> .	<i>A. pallidus</i> .	<i>A. splendens</i> .	<i>A. subpictus</i> .	<i>A. tessellatus</i> .	<i>A. vagus</i> .	<i>A. varuna</i> .
February 1940	..	6.0	..	..	6.0	2.0	..	..	..	7.0	..	3.0	..	..	..	3.0	..	..	..	..	..	..	..
March "	..	3.8	..	..	4.3	7.0	3.0	1.0	..	1.0	1.0	3.0	..	..	..	7.0	..	..	..	..	1.0	2.0	..
April "	..	5.5	..	..	6.0	4.0	3.7	2.0	2.0	2.0	2.3	..	..	..	1.0	9.9	..	..	..	3.3	1.0	3.0	..
May "	..	3.0	..	..	4.0	4.5	10.0	..	..	3.0	..	4.5	..	2.5	..	7.0	..	..	..	2.0	..	..	..
June "	1.0	4.1	..	..	..	8.0	6.3	..	6.0	..	..	4.0	..	10.5	..	12.9	..	..	1.0	..	..	2.3	..
July "	..	9.9	..	..	4.5	..	23.4	..	..	..	..	6.0	..	5.0	..	10.0	..	..	..	59.0	..	..	..
August "	..	7.2	..	..	3.0	55.0	10.4	..	..	..	..	8.0	..	1.0	..	7.6	..	2.0	..	..	..	..	..
September "	..	1.3	..	..	1.5	27.0	13.0	..	..	..	2.0	1.0	5.3	7.0	..	8.0	..	..	..	..	..	..	..
October "	..	2.0	..	..	1.4	44.5	8.7	..	..	..	2.0	2.0	2.0	15.5	..	8.1	..	..	..	..	..	60.0	..
November "	..	4.0	..	..	2.5	7.5	6.5	..	..	..	..	..	..	..	..	4.3	..	..	..	20.0	..	4.6	..
December "	..	2.2	..	..	2.0	2.0	1.0	..	1.5	1.0	20.0	8.0	..	2.0	..	4.3	..	..	3.0	1.5	..	1.0	..
January 1941	..	4.7	..	..	3.3	16.0	6.3	..	..	1.0	1.0	2.5	4.0	..	..	4.0	..	..	..	5.0	3.0	..	..
Average per 15 minutes.	1.0	4.5	..	..	3.5	16.1	8.4	1.5	3.2	2.5	4.7	4.2	3.8	6.2	1.0	7.3	..	..	2.0	15.1	1.7	12.2	..



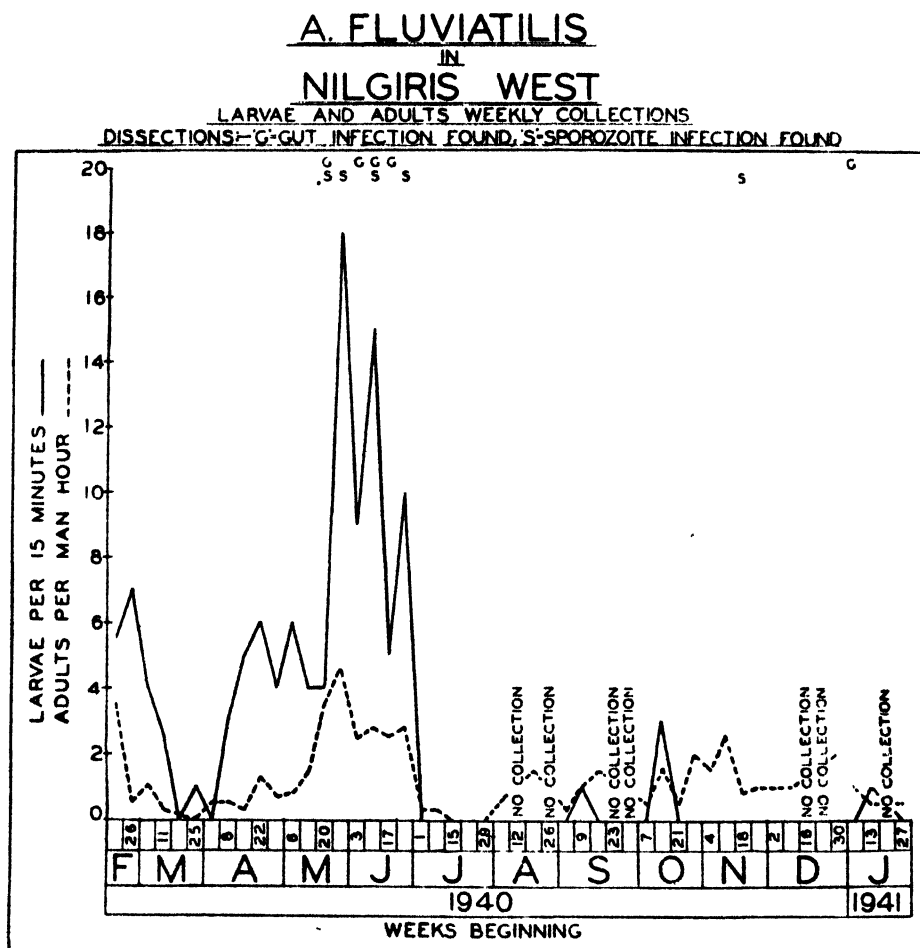
TABLE IX.

A. *fluviatilis* larvæ and adults collected in Nilgiris west and east, February 1940 to January 1941 (larvæ per 15 minutes and adults per man-hour collecting time).

Week beginning	NILGIRIS WEST.		NILGIRIS EAST.	
	Larvæ.	Adults.	Larvæ.	Adults.
February 19, 1940	5.5	3.5	0.0	26.1
" 26, "	7.0	0.5	0.0	30.8
March 4, "	4.0	1.0	0.0	13.5
" 11, "	2.5	0.3	0.0	14.0
" 18, "	0.0	0.1	5.0	14.9
" 25, "	1.0	0.0	1.0	17.4
April 1, "	0.0	0.5	1.0	16.3
" 8, "	3.0	0.5	0.0	24.7
" 15, "	5.0	0.3	5.0	17.6
" 22, "	6.0	1.3	3.5	36.8
" 29, "	4.0	0.7	0.0	26.0
May 6, "	6.0	0.8	0.0	20.3
" 13, "	4.0	1.5	0.0	20.5
" 20, "	4.0	3.5	8.0	35.4
" 27, "	18.0	4.6	12.0	18.0
June 3, "	9.0	2.5	5.0	20.0
" 10, "	15.0	2.8	2.0	27.0
" 17, "	5.0	2.5	4.5	34.0
" 24, "	10.0	2.8	11.0	31.0
July 1, "	0.0	0.3	23.0	22.5
" 8, "	0.0	0.3	38.0	17.0
" 15, "	0.0	0.0	6.0	23.3
" 22, "	0.0	0.0	11.0	30.0
" 29, "	0.0	0.0	10.0	26.8
August 5, "	0.0	0.6	10.0	26.0
" 12, "	Not collected.		4.0	23.3
" 19, "	0.0	1.5	5.0	11.8
" 26, "	Not collected.		17.5	15.8
September 2, "	0.0	0.3	12.0	7.9
" 9, "	1.0	1.0	8.0	20.3
" 16, "	0.0	1.5	20.0	28.5
" 23, "	Not collected.		0.0	15.8
" 30, "	Not collected.		2.0	11.3
October 7, "	0.0	0.5	2.0	12.8
" 14, "	3.0	1.6	13.0	11.0
" 21, "	0.0	0.5	11.0	12.3
" 28, "	0.0	2.0	0.0	12.0
November 4, "	0.0	1.5	0.0	15.0
" 11, "	0.0	2.0	8.0	15.5
" 18, "	0.0	0.8	0.0	15.0
" 25, "	0.0	1.0	0.0	16.2
December 2, "	0.0	1.0	1.0	20.5
" 9, "	0.0	1.0	0.0	31.5
" 16, "	Not collected.		0.0	23.0
" 23, "	Not collected.		Not collected.	
" 30, "	0.0	2.0	11.0	9.8
January 6, 1941	0.0	1.0	6.0	10.8
" 13, "	1.0	0.5	0.0	21.5
" 20, "	Not collected.		Not collected.	
" 27, "	0.0	0.5	2.0	14.0



## CHART 2.

Incidence of *A. fluviatilis* and positive dissections, Nilgiris west.

high average temperatures) *A. fluviatilis* may appear at levels up to 5,500 feet. Certain estates at this level have had outbreaks of malaria apparently due to *A. fluviatilis* (verbal communication from Dr. G. C. Ramsay). It is also possible that houses near the edge of a cliff may be invaded by *A. fluviatilis* which has flown or been carried up from a stream 1,000 to 1,500 feet below.

Data regarding collections of adult anophelines are given in Tables XI to XV. In the first two tables, figures for Nilgiris west and east, respectively, are given by species and months, expressed as totals taken per man-hour of collecting. Seasonal trends will be noted, but it is only necessary here to point out that the seasonal distribution and density of *A. fluviatilis*, the vector, were markedly different in Nilgiris east than in the west (Charts 2 and 3). In Nilgiris east, this



CHART 3.

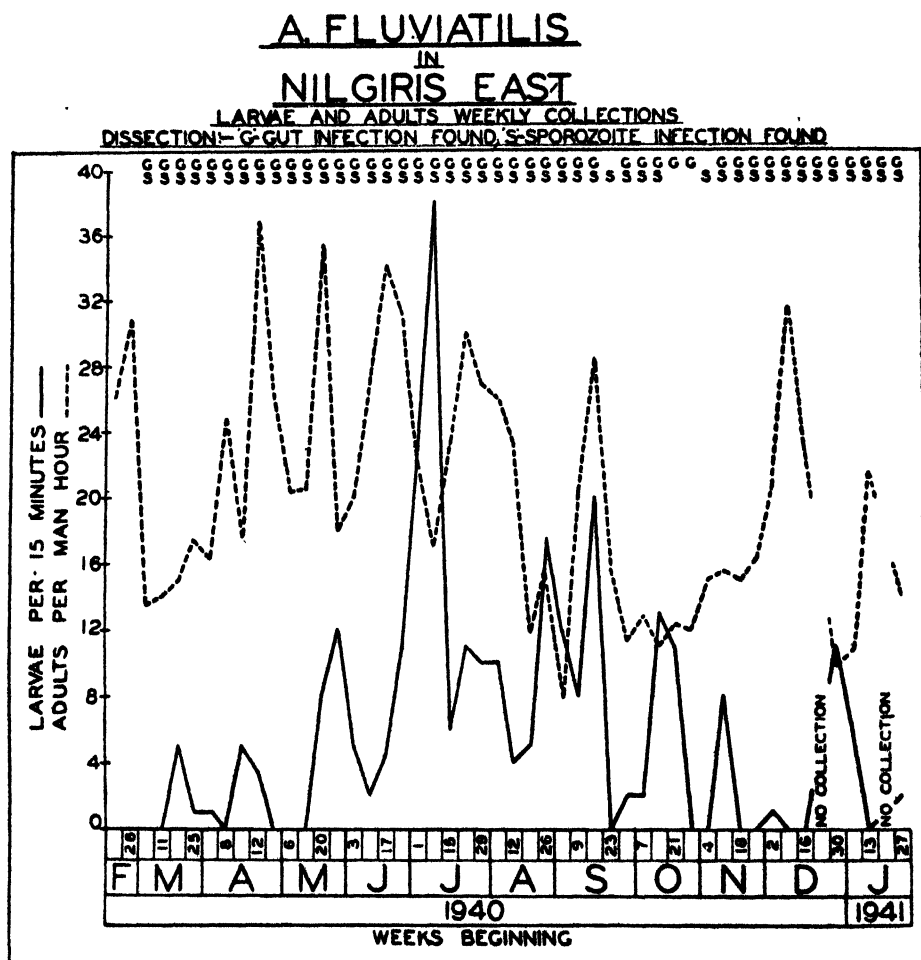
Incidence of *A. fluviatilis* and positive dissections, Nilgiris east.



TABLE X.

*Anopheles* larvæ collected at various altitudes in the Nilgiris area from February 1940 to January 1941.

Species.	ALTITUDE (IN FEET).		
	Up to 1,999	2,000 to 3,999	4,000 and above
<i>A. aconitus</i> .. ..	1	36	0
<i>A. aitkeni</i> .. ..	278	188	1,495
<i>A. annandalei</i> var. <i>interruptus</i>	0	4	0
<i>A. annularis</i> .. ..	0	79	14
<i>A. barbirostris</i> .. ..	102	1,015	209
<i>A. culicifacies</i> .. ..	405	29	16
<i>A. fluviatilis</i> .. ..	502	308	0
<i>A. gigas</i> type .. ..	1	5	444
<i>A. gigas</i> var. <i>simlensis</i> ..	7	14	231
<i>A. hyrcanus</i> var. <i>nigerrimus</i> ..	20	398	21
<i>A. jamesi</i> .. ..	23	339	0
<i>A. jeyporiensis</i> .. ..	73	602	108
<i>A. karwari</i> .. ..	20	28	0
<i>A. leucosphyrus</i> .. ..	0	96	10
<i>A. lindesayi</i> var. <i>nilgircus</i> ..	0	2	116
<i>A. maculatus</i> .. ..	406	1,369	900
<i>A. majidi</i> .. ..	0	43	0
<i>A. pallidus</i> .. ..	0	5	0
<i>A. splendidus</i> .. ..	6	9	5
<i>A. subpictus</i> .. ..	158	16	2
<i>A. tessellatus</i> .. ..	5	..	0
<i>A. vagus</i> .. ..	94	81	25
<i>A. varuna</i> .. ..	0	26	0
TOTAL ..	2,101.	4,692	3,596



TABLE XI.

*Anopheles* adults collected, Nilgiris west, February 1940 to January 1941 (monthly totals by man-hour).

Month and year.	<i>A. aconitus</i> .	<i>A. aikeni</i> type.	<i>A. annandalei</i> var.	<i>A. annularis</i> .	<i>A. barbroestrus</i> .	<i>A. culicifacies</i> .	<i>A. fluviatilis</i> .	<i>A. gigns</i> type.	<i>A. gigns</i> var. <i>simlensis</i> .	<i>A. hyrcanus</i> var. <i>nigerrimus</i> .	<i>A. jamesi</i> .	<i>A. jeyporensis</i> .	<i>A. karwari</i> .	<i>A. leucosphyrus</i> .	<i>A. lindesayi</i> var.	<i>A. maculatus</i> .	<i>A. maidi</i> .	<i>A. pallidus</i> .	<i>A. splendens</i> .	<i>A. subpictus</i> .	<i>A. tessellatus</i> .	<i>A. vagus</i> .	<i>A. varuna</i> .
February 1940	..	..	..	..	..	..	1.9	..	..	..	0.8	4.8	..	..	..	1.1	0.8	..	0.5	0.1	..	..	..
March	..	..	..	..	..	0.4	0.5	..	..	..	0.9	3.5	..	..	..	0.4	0.4	..	0.1	..	..	0.3	..
April	..	..	..	..	..	0.1	0.8	..	..	..	0.3	5.9	..	..	..	0.7	0.4	..	0.4	0.6	..	..	..
May	..	..	..	..	..	0.3	2.7	..	..	0.1	..	3.8	..	..	..	..	0.2	..	0.3	0.4	..	..	..
June	0.1	..	..	..	0.3	0.4	2.5	..	..	0.2	2.5	4.6	..	..	..	0.1	0.4	..	..	0.4	..	1.2	0.1
July	..	..	..	..	0.2	..	0.1	..	..	..	6.6	6.7	..	..	..	0.1	0.4	..	..	0.1	..	0.1	0.1
August	..	..	..	..	2.0	..	2.5	..	..	..	5.4	5.6	..	..	..	0.4	1.0	..	..	..	..	1.4	..
September	0.1	..	..	..	0.1	..	0.9	..	..	0.3	2.0	4.0	0.1	..	..	0.3	0.1	..	0.1	..	..	4.1	..
October	..	..	..	..	..	0.2	1.1	..	..	0.1	0.2	7.0	1.2	..	..	0.3	0.1	..	0.1	6.2	..	3.7	..
November	0.1	..	..	0.1	0.1	0.4	1.2	..	..	0.5	0.3	4.7	0.1	..	..	..	1.0	..	..	6.3	..	5.2	0.3
December	0.1	..	..	..	..	0.4	0.8	..	..	..	1.8	7.1	0.1	..	..	0.1	0.4	..	0.1	3.6	..	1.8	..
January 1941	..	..	..	..	..	0.2	0.7	..	..	..	1.7	10.5	..	..	..	..	0.9	..	0.3	3.9	..	2.7	..



TABLE XII.  
*Anopheles* adults collected, Nilgiris east, February 1940 to January 1941 (monthly totals by man-hour).

Month and year.	<i>A. aconitus</i> .	<i>A. ailanti</i> type.	<i>A. annandalei</i> var. <i>interruptus</i> .	<i>A. annularis</i> .	<i>A. barbrosistris</i> .	<i>A. culicifacies</i> .	<i>A. fluviatilis</i> .	<i>A. gigas</i> type.	<i>A. gigas</i> var. <i>simlensis</i> .	<i>A. hyrcanus</i> var. <i>nigerrimus</i> .	<i>A. jamesti</i> .	<i>A. jeyporiensis</i> .	<i>A. karwari</i> .	<i>A. leucosphyrus</i> .	<i>A. lindesayi</i> var. <i>nilgiris</i> .	<i>A. maculatus</i> .	<i>A. mayidi</i> .	<i>A. pallidus</i> .	<i>A. splendens</i> .	<i>A. subpictus</i> .	<i>A. tessellatus</i> .	<i>A. vagus</i> .	<i>A. varuna</i> .
February 1940	..	..	..	..	..	0.2	19.0	..	..	..	0.2	0.2	..	..	..	0.2	..	..	0.2	..	..	..	..
March	..	..	..	..	..	0.6	14.9	..	..	0.1	0.1	0.4	..	..	..	0.1	..	..	..	0.4	0.2	1.8	0.1
April	..	..	..	..	..	0.6	24.5	..	..	..	0.1	0.8	..	..	..	0.2	..	..	..	1.6	0.1	0.6	..
May	..	..	..	..	..	0.2	23.0	..	..	..	0.1	0.6	..	..	..	0.1	..	..	..	..	0.1	0.4	0.1
June	..	..	..	..	..	..	28.5	..	..	..	..	..	..	..	..	..	..	..	..	0.5	..	1.4	0.2
July	..	0.1	..	..	..	..	23.9	..	..	..	..	..	..	..	..	0.1	..	..	..	..	..	0.9	0.1
August	..	..	..	..	..	..	19.2	..	..	..	..	..	..	..	..	..	..	..	..	..	..	1.7	..
September	..	..	..	..	0.1	0.4	16.8	..	..	0.3	0.1	0.3	0.1	..	..	0.2	..	..	..	3.5	0.4	4.8	..
October	..	..	..	..	..	0.1	12.0	..	..	0.5	0.1	..	0.1	..	..	..	..	..	..	13.1	0.2	13.8	..
November	..	..	..	..	..	..	15.5	..	..	0.8	0.3	0.4	..	..	..	..	..	..	0.1	3.2	0.1	3.4	0.3
December	..	..	..	..	..	0.1	21.2	..	..	0.5	0.1	0.9	..	..	..	0.1	..	..	..	0.5	0.1	1.6	..
January 1941	..	..	..	..	..	0.3	15.7	..	..	0.3	..	0.2	..	..	..	0.4	..	0.1	..	0.2	0.2	0.3	..



often found on utensils or articles leaning against the wall. Species like *A. jamesi*, *A. jeyporiensis*, *A. subpictus* and *A. vagus* were more commonly taken

TABLE XIII. ,  
*Anopheles* adults, by resting places, Nilgiris west, February 1940 to January 1941.

Species.	HUMAN DWELLINGS.			MIXED DWELLINGS.			ANIMAL DWELLINGS.			TOTAL.		
	M.	F.	T.	M.	F.	T.	M.	F.	T.	M.	F.	T.
<i>A. aconitus</i> ..	..	1	1	..	1	1	..	2	2	..	4	4
<i>A. aitkeni</i> ..	..	..	..	..	..	..	..	..	..	..	..	..
<i>A. annularis</i> ..	..	..	..	..	..	..	..	1	1	..	1	1
<i>A. barbirostris</i> ..	..	1	1	..	2	2	..	15	15	..	18	18
<i>A. culicifacies</i> ..	..	3	3	..	..	..	..	23	23	..	26	26
<i>A. fluviatilis</i> ..	4	99	103	..	5	5	2	19	21	6	123	129
<i>A. gigas</i> ..	..	..	..	..	..	..	..	..	..	..	..	..
<i>A. hyrcanus</i> ..	..	2	2	..	..	..	..	16	16	..	18	18
<i>A. jamesi</i> ..	..	10	10	..	7	7	1	264	265	1	281	282
<i>A. jeyporiensis</i> ..	..	53	53	..	56	56	8	519	527	8	628	636
<i>A. karwari</i> ..	..	10	10	..	1	1	..	..	..	..	11	11
<i>A. maculatus</i> ..	..	3	3	..	1	1	..	37	37	..	41	41
<i>A. majidi</i> ..	..	2	2	..	1	1	..	50	50	..	53	53
<i>A. splendidus</i> ..	..	..	..	..	..	..	..	13	13	..	13	13
<i>A. subpictus</i> ..	..	23	23	..	12	12	..	106	106	..	141	141
<i>A. tessellatus</i> ..	..	..	..	..	..	..	..	..	..	..	..	..
<i>A. vagus</i> ..	..	22	22	..	19	19	..	147	147	..	188	188
<i>A. varuna</i> ..	..	2	2	..	..	..	..	2	2	..	4	4
<i>A. subpictus</i> or <i>A. vagus.</i>	12	..	12	5	..	5	8	..	8	25	..	25
TOTAL ..	16	231	247	5	105	110	19	1,214	1,233	40	1,550	1,590

in cowsheds. Larvæ of *A. barbirostris* were taken in fairly large numbers, but adults were seldom encountered in routine collections. This same dissociation



was noted by Covell and Harbhagwan (*loc. cit.*) in the Wynaad, and by Russell and Rao (1941) in Pattukkottai.

TABLE XIV.

*Anopheles* adults, by resting places, Nilgiris east, February 1940 to January 1941.

Species.	HUMAN DWELLINGS.			MIXED DWELLINGS.			ANIMAL DWELLINGS.			TOTAL.		
	M.	F.	T.	M.	F.	T.	M.	F.	T.	M.	F.	T.
<i>A. aconitus</i> ..	..	..	..	..	..	..	..	..	..	..	..	..
<i>A. aitkeni</i> ..	..	1	1	..	..	..	..	..	..	..	1	1
<i>A. annularis</i> ..	..	..	..	..	..	..	..	..	..	..	..	..
<i>A. barbirostris</i> ..	..	..	..	..	..	..	..	1	1	..	1	1
<i>A. culicifacies</i> ..	..	17	17	..	12	12	..	2	2	..	31	31
<i>A. fluviatilis</i> ..	139	3,759	3,898	16	256	272	..	12	12	155	4,027	4,182
<i>A. gigas</i> ..	..	..	..	..	..	..	..	..	..	..	..	..
<i>A. hyrcanus</i> ..	..	1	1	..	..	..	..	39	39	..	40	40
<i>A. jamesi</i> ..	..	..	..	..	..	..	..	14	14	..	14	14
<i>A. jeyporiensis</i> ..	1	33	34	..	2	2	..	26	26	1	61	62
<i>A. karwari</i> ..	..	..	..	..	..	..	..	2	2	..	2	2
<i>A. maculatus</i> ..	..	6	6	..	2	2	..	9	9	..	17	17
<i>A. majidi</i> ..	..	..	..	..	..	..	..	1	1	..	1	1
<i>A. splendidus</i> ..	..	1	1	..	..	..	..	..	..	..	1	1
<i>A. subpictus</i> ..	..	149	149	..	204	204	..	19	19	..	372	372
<i>A. tessellatus</i> ..	..	5	5	..	..	..	..	19	19	..	24	24
<i>A. vagus</i> ..	..	459	459	..	77	77	..	28	28	..	564	564
<i>A. varuna</i> ..	1	9	10	..	..	..	..	1	1	1	10	11
<i>A. subpictus</i> or <i>A. vagus.</i>	93	..	93	8	..	8	2	..	2	103	..	103
TOTAL ..	234	4,440	4,674	24	553	577	2	173	175	260	5,166	5,426

No precipitin tests were made, but it should be noted that Covell and Harbhagwan (*loc. cit.*), in the neighbouring Wynaad, in 1,681 positive tests with



TABLE XV.

*Anopheles* adults, by resting places, Nilgiris central (Coonoor), February 1940 to January 1941.

Species.	HUMAN DWELLINGS.			MIXED DWELLINGS.			ANIMAL DWELLINGS.			TOTAL.		
	M.	F.	T.	M.	F.	T.	M.	F.	T.	M.	F.	T.
<i>A. aconitus</i> ..	..	..	..	..	..	..	..	..	..	..	..	..
<i>A. aitkeni</i> ..	..	..	..	..	..	..	..	..	..	..	4	4*
<i>A. annularis</i> ..	..	..	..	..	..	..	..	..	..	..	..	..
<i>A. barbirostris</i> ..	..	..	..	..	..	..	..	..	..	..	..	..
<i>A. culicifacies</i> ..	..	1	1	..	..	..	..	..	..	..	1	1
<i>A. fluviatilis</i> ..	..	..	..	..	..	..	..	..	..	..	..	..
<i>A. gigas</i> ..	..	..	..	..	..	..	..	2	2	..	2	2
<i>A. hyrcanus</i> ..	..	..	..	..	..	..	..	..	..	..	..	..
<i>A. jamesi</i> ..	..	..	..	..	..	..	..	..	..	..	..	..
<i>A. jeyporiensis</i> ..	..	5	5	..	..	..	..	6	6	..	11	11
<i>A. karwari</i> ..	..	..	..	..	..	..	..	..	..	..	..	..
<i>A. maculatus</i> ..	..	..	..	..	..	..	..	24	24	..	24	24
<i>A. majidi</i> ..	..	..	..	..	..	..	..	..	..	..	..	..
<i>A. splendidus</i> ..	..	..	..	..	..	..	..	5	5	..	5	5
<i>A. subpictus</i> ..	..	1	1	..	..	..	..	39	39	..	40	40
<i>A. tessellatus</i> ..	..	..	..	..	..	..	..	1	1	..	1	1
<i>A. vagus</i> ..	..	6	6	..	..	..	..	48	48	..	54	54
<i>A. varuna</i> ..	..	..	..	..	..	..	..	..	..	..	..	..
<i>A. subpictus</i> or <i>A. vagus.</i>	..	..	..	..	..	..	..	..	..	..	..	..
TOTAL ..	..	13	13	..	..	..	..	125	125	..	142	142

\* Four *A. aitkeni* were collected while biting mosquito collector in jungle near a stream.

*A. fluviatilis* found no less than 97 per cent to have fed on human blood. There is no reason to believe that the results would have been significantly different in the Nilgiris, where conditions as regards human and bovine population, and general habits of *A. fluviatilis*, are similar.



TABLE XVI.

*Dissections of Anopheles adults, Nilgiris, February 1940 to January 1941.*  
*All species years totals infection rate.*

Species.	Number dissected.	POSITIVE.		Gut dissected.	POSITIVE.		Glands dissected.	POSITIVE.	
		Num-ber.	Per cent.		Num-ber.	Per cent.		Num-ber.	Per cent.
<i>A. aconitus</i> ..	2	0	..	2	0	..	2	0	..
<i>A. annularis</i> ..	1	0	..	1	0	..	1	0	..
<i>A. barbirostris</i> ..	7	0	..	7	0	..	7	0	..
<i>A. culicifacies</i> ..	37	0	..	37	0	..	37	0	..
<i>A. fluviatilis</i> ..	2,580	447	17.3	2,580	252	9.7	2,580	262	10.1
<i>A. gigas</i> ..	5	0	..	5	0	..	5	0	..
<i>A. hyrcanus</i> ..	38	0	..	38	0	..	38	0	..
<i>A. jamesi</i> ..	94	0	..	94	0	..	94	0	..
<i>A. jeyporensis</i> ..	377	0	..	377	0	..	377	0	..
<i>A. karwari</i> ..	8	0	..	8	0	..	8	0	..
<i>A. maculatus</i> ..	39	0	..	39	0	..	39	0	..
<i>A. majidi</i> ..	28	0	..	28	0	..	28	0	..
<i>A. splendidus</i> ..	12	0	..	12	0	..	12	0	..
<i>A. subpictus</i> ..	387	0	..	387	0	..	387	0	..
<i>A. tessellatus</i> ..	12	0	..	12	0	..	12	0	..
<i>A. vagus</i> ..	464	0	..	464	0	..	464	0	..
<i>A. varuna</i> ..	4	0	..	4	0	..	4	0	..
TOTAL ..	4,005	447	10.9	4,005	252	6.1	4,005	262	6.3

Dissection data are given in Tables XVI and XVII (Charts 2 and 3). It will be noted that of a total of 2,580 *A. fluviatilis* dissected, no fewer than 447, or 17.3 per cent, were found infected. The oöcyst and sporozoite indices were 9.7 and 10.1 per cent, respectively. Among 1,515 dissections of other species, collected in the same places, none was found to be infected, but the numbers by species dissected were usually too small to be of definite significance. However, in the presence of a relatively high gametocyte index these negative results have some value. It seems safe to say, in view of the fact that our results confirm those of Covell and Harbhagwan (*loc. cit.*), that the only vector of any importance in this area is *A. fluviatilis*.



Table XVII gives dissection results by weeks for *A. fluviatilis* (Charts 2 and 3). It is interesting that, with two exceptions (November 18 and January 6), the only infections in Nilgiris west were found in the months of May and June, whereas in Nilgiris east (Kallar) infected specimens were found every week from March 4, 1940 to January 20, 1941, inclusive. There must be few places in the world where such a record could be made. In this hyperendemic area weekly sporozoite indices ranged from 1.5 (June 24) to 34.8 per cent (June 3), and only in two weeks were no sporozoites found (October 21 and 28). Dissecting *A. fluviatilis* collected in Sultan's Battery, in the Wynaad, Covell and Harbhagwan (*loc. cit.*) found sporozoite indices ranging from 1.0 to 14.5 per cent and total infection indices from 5.4 to 50.0 per cent, by monthly totals. Surely, in view of these very high infection rates, fully confirmed by our dissections, and considering its exceptionally marked preference for human blood, *A. fluviatilis* must be rated as one of the most efficient of all malaria vectors.

It is interesting to contrast the infections in *A. fluviatilis* with our findings in Pattukkottai Taluk relative to *A. culicifacies*. As reported by Russell and Rao (1940), the sporozoite index for this species in 13,145 dissections was only 0.61 per cent. Total monthly infection indices ranged from 0.00 to 0.28 per cent. Yet, by virtue of its density, *A. culicifacies* succeeds in maintaining in that taluk endemic spleen indices ranging from 20 to 80 per cent. On the basis of larval collections per 15 minutes and adult collections per man-hour, it is apparent

TABLE XVII.

Dissections of *A. fluviatilis*, by weeks, February 1940 to January 1941.

Weeks beginning with Monday, Date.	NILGIRIS WEST.			NILGIRIS EAST.			TOTAL.		
	Number dissected.	Per cent with		Number dissected.	Per cent with		Number dissected.	Per cent with	
		Oöcysts.	Sporozoites.		Oöcysts.	Sporozoites.		Oöcysts.	Sporozoites.
February 19, 1940	..	..	..	18	..	..	18	..	..
" 26, "	5	..	..	34	..	..	39	..	..
March 4, "	..	..	..	63	7.9	3.2	63	7.9	3.2
" 12, "	1	..	..	21	14.3	4.8	22	13.6	4.6
" 18, "	3	..	..	59	20.4	13.6	62	19.3	12.9
" 25, "	1	..	..	30	13.3	10.0	31	12.9	9.7
April 1, "	..	..	..	35	22.9	8.6	35	22.9	8.6
" 8, "	..	..	..	41	7.3	2.4	41	7.3	2.4
" 15, "	2	..	..	57	12.3	9.1	59	11.9	8.5
" 22, "	2	..	..	84	11.9	5.9	86	11.6	5.8
" 29, "	..	..	..	55	3.5	16.3	55	3.5	16.3
May 6, "	..	..	..	62	6.5	12.9	62	6.5	12.9
" 13, "	..	..	..	63	11.1	14.3	63	11.1	14.3
" 20, "	9	11.1	33.3	50	10.0	12.0	59	10.2	15.3
" 27, "	3	..	33.3	39	7.7	10.3	42	7.2	11.9



TABLE XVII—*contd.*

Weeks beginning with Monday, Date.		NILGIRIS WEST.			NILGIRIS EAST.			TOTAL.		
		Number dissected.	Per cent with		Number dissected.	Per cent with		Number dissected.	Per cent with	
			Oöcysts.	Sporozoites.		Oöcysts.	Sporozoites.		Oöcysts.	Sporozoites.
June	3, 1940	10	10.0	..	46	32.6	34.8	56	28.6	28.6
"	10, "	12	16.7	25.0	70	5.7	5.7	82	7.3	8.5
"	17, "	4	25.0	..	56	19.7	17.8	60	20.0	16.7
"	24, "	8	..	25.0	139	9.3	1.5	147	8.8	2.8
July	1, "	1	..	..	77	7.8	12.9	78	7.7	12.8
"	8, "	6	..	..	81	4.9	7.4	87	4.6	6.9
"	15, "	..	..	..	26	11.6	23.2	26	11.6	23.2
"	22, "	..	..	..	29	3.5	20.7	29	3.5	20.7
"	29, "	..	..	..	126	3.2	11.9	126	3.2	11.9
August	5, "	..	..	..	66	10.6	12.1	66	10.6	12.1
"	12, "	1	..	..	68	16.2	7.4	69	16.1	7.3
"	19, "	..	..	..	75	4.0	8.0	75	4.0	8.0
"	26, "	..	..	..	71	2.8	8.4	71	2.8	8.4
September	2, "	1	..	..	38	5.3	10.5	39	5.1	10.3
"	9, "	..	..	..	50	8.0	8.0	50	8.0	8.0
"	16, "	1	..	..	44	4.5	4.5	45	4.4	4.4
"	23, "	..	..	..	17	..	5.9	17	..	5.9
"	30, "	..	..	..	79	5.1	7.8	79	5.1	7.8
October	7, "	1	..	..	34	14.7	17.6	35	14.3	17.2
"	14, "	..	..	..	24	4.2	12.5	24	4.2	12.5
"	21, "	..	..	..	28	7.1	..	28	7.1	..
"	28, "	..	..	..	6	33.3	..	6	33.3	..
November	4, "	2	..	..	26	..	3.9	28	3.6	..
"	11, "	2	..	..	30	10.0	3.3	32	9.4	3.1
"	18, "	3	..	33.3	20	20.0	15.0	23	17.4	17.4
"	25, "	4	..	..	62	16.1	11.3	66	15.2	10.6
December	2, "	1	..	..	67	13.4	8.9	68	13.3	8.8
"	9, "	..	..	..	36	8.5	13.9	36	8.5	13.9
"	16, "	1	..	..	53	15.1	13.2	54	15.0	13.1
"	23, "	1	..	..	116	12.1	11.2	117	11.9	11.1
"	30, "	..	..	..	31	16.1	6.5	31	16.5	6.5
January	6, 1941	4	25.0	..	24	20.8	16.7	28	21.4	14.3
"	13, "	1	..	..	13	7.7	7.7	14	7.2	7.2
"	20, "	1	..	..	50	4.0	4.0	51	3.9	3.9
"	27, "	..	..	..	..	..	..	..	..	..
TOTAL ..		91	6.6	10.9	2,489	9.8	9.7	2,580	9.7	10.1

that *A. fluviatilis* had a much lower density in the Nilgiris than *A. culicifacies* in Pattukkottai.

Other reports of infected *A. fluviatilis* (formerly called *A. listonii*) are those of Horne (1914), who found two infections in 30 dissections, or 6.6 per cent, in the Wynaad; Perry (1914), who found 1.8 per cent gland infections in 229



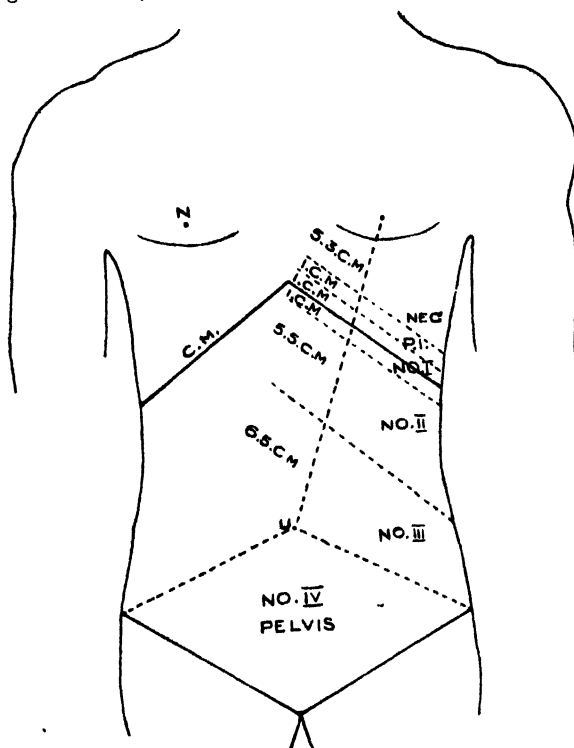
dissections in the Jeypore Hill Tracts; Chalam (1923), who found 3·8 per cent gland infections in 315 dissections in Assam (these may have been *A. minimus* and not *A. fluviatilis*); King and Iyer (1929), who found 3·9 per cent gut infections in 77 dissections in Mopad, Madras Presidency; Mathew (1939), who found 23·7 per cent gland and 13·0 per cent gut infections in 2,602 dissections; and Measham (1939 p. 25), who reported infection indices of 10·12 per cent in the Anamallais and 10·57 per cent in the Wynaad, both in Madras Presidency. All but the last report are cited by Covell (1927; 1937).

### SPLEEN AND PARASITE INDICES.

Data regarding spleen and parasite indices are shown in Tables XVIII to XXVI. Splenic enlargement was classified as follows (Text-fig. 3):—

Text-figure 3.

Diagram showing method of classifying splenic enlargement.



Negative = Spleen not palpable even on deep inspiration.

P.I. = Apex of spleen palpable on inspiration but not palpable unless subject inspires.

I. = Apex palpable near costal margin without aid of inspiration, but apex not extending more than one centimetre below costal margin. (If apex is above costal margin, it is not usually palpable more than one centimetre above it.)



TABLE XVIII.

*Spleen palpations, Nilgiris area, school children aged 2 to 14, 1940.*

Place.	Date, 1940.	Number examined.	PALPABLE.		SPLEEN SIZES (TOTAL).				
			Num-ber.	Per-cent	P.I.	1	2	3	4
Devala ..	Feb. 23	40	19	47.5	0	12	4	2	1
Devala ..	May 21	74	27	36.7	4	13	5	2	3
Devala ..	Dec. 13	52	25	48.1	1	13	5	3	3
Deverashola ..	Feb. 23	35	11	31.4	1	10	0	0	0
Gudalur ..	Feb. 16	69	29	42.0	4	14	9	1	1
Gudalur ..	Aug. 29	104	54	51.9	10	22	15	3	4
Massinigudi ..	Feb. 16	16	8	50.0	1	3	3	1	0
Massinigudi ..	Aug. 29	26	20	76.9	4	3	5	2	6
Nellakkotta ..	Feb. 23	20	5	25.0	1	4	0	0	0
Singara ..	Feb. 16	30	15	50.0	5	7	1	2	0
Teppakkadu ..	Feb. 16	10	8	80.0	0	1	3	3	1
Nilgiris west total		476	221	46.5	31	102	50	19	19
Benhope ..	May 15	27	13	48.1	7	6	0	0	0
Burliar ..	Apr. 12	31	27	87.1	0	11	9	3	4
Burliar ..	Oct. 13	36	26	72.2	0	9	12	4	1
Kallar ..	Mar. 27	22	22	100.0	0	5	13	4	0
Kallar ..	June 1	24	17	70.1	0	5	2	5	5
Kallar ..	Oct. 27	20	20	100.0	0	2	15	3	0
Nellithurai ..	Aug. 29	48	30	62.5	3	3	11	5	8
Nilgiris east total		208	155	84.5	10	41	62	24	18
Coonoor ..	March and April	706	7	0.9	4	3	0	0	0
Dayanur ..	Aug. 29	28	0	0.0	0	0	0	0	0
Devarshola ..	May 8	56	1	1.9	1	0	0	0	0
Jekkneri ..	Feb. 14	86	15	17.4	4	11	0	0	0
Kilinjada ..	Feb. 26	40	15	37.5	2	9	2	2	0
Kilkotagiri ..	Aug. 16	35	1	2.9	0	1	0	0	0
Kilkunda ..	May 8	25	2	8.0	0	2	0	0	0
Kodanad ..	Aug. 16	47	4	8.5	0	2	1	1	0
Kolakambe ..	Feb. 12	77	18	23.4	4	6	4	3	1
Konakkarai ..	Aug. 16	67	2	2.9	0	2	0	0	0
Melkunda ..	May 8	55	0	0.0	0	0	0	0	0
Mettupalayam (school)	Feb. 22	115	4	3.5	2	2	0	0	0
Mettupalayam (near bridge).	July 28	87	0	0.0	0	0	0	0	0
Mettupalayam (potter's st.).	July 31	27	1	3.7	0	1	0	0	0
Neduvattam ..	Feb. 27	54	7	12.9	0	6	0	1	0
Nirgundi ..	Aug. 16	34	2	5.9	0	1	1	0	0
Nonsuch Estate	May 26	187	10	5.3	3	7	0	0	0
Prospect Estate	May 31	27	2	7.4	0	1	1	0	0
Pungampalayam	Aug. 29	51	4	7.9	2	1	1	0	0
Selas ..	Feb. 19	30	4	13.0	2	2	0	0	0
Sholurmattam	Aug. 16	36	4	11.1	0	4	0	0	0
Tekkampatti ..	Aug. 29	52	5	9.7	1	1	2	1	0
Tholampalayam	Aug. 29	87	6	6.9	2	4	0	0	0
Vellingodu ..	Aug. 29	32	1	3.1	0	1	0	0	0
Total (excluding Nilgiris west and east).	=	2,041	115	5.6	27	67	12	8	1



- II. = Apex palpable more than one centimetre below costal margin, but not more than half-way between this margin and the umbilicus.
- III. = Apex palpable more than half-way from costal margin to the umbilicus, but not below latter.
- IV. = Apex of spleen palpable below umbilicus, in pelvis.

Whenever possible children were examined when lying on their backs, knees and hips flexed. Sometimes circumstances made it necessary to examine children when standing. As noted by Russell, Menon and Rao (1938), when the same children were examined in both positions very little difference was noted, although an occasional P.I. spleen, evident when the child was lying down, was not palpable when the child was standing. Generally, in our experience, enlarged spleens, unless very large, do not drop lower by gravity when the subject stands. They tend to be lifted a little higher, presumably by their anatomical attachments. We have not noted more than 0.5 cm. difference in any case so far tested.

#### NILGIRIS WEST.

In Table XVIII, all spleen examinations during the course of the survey have been listed. It will be noted that in Nilgiris west the average index was 46.5 per cent, in Nilgiris east 84.5 per cent, and in other areas only 5.6 per cent.

Previous reports in the Devala-Gudalur area are those of Horne (1913) and Viswanathan (1936). Horne's report may be summarized as follows:—

*Spleen rates found in Nilgiris west by Horne (1913), May and June 1913.*

Place.	Number examined.	PALPABLE.		SPLEEN SIZES.*				
		Number.	Per cent	V.	IV.	III.	II.	I.
Nella† .. ..	17	6	35.3	11	6	..	..	..
Devarshola† .. ..	19	5	26.3	14	4	1	..	..
Gudalur† .. ..	50	8	16.0	42	3	5	..	..
Devala .. ..	Children	15	12	80.0	3	1	5	3
	Adults	11	7	63.6	..	..	1	5
Pondalur .. ..	Children	23	13	56.5	10	3	5	4
	Adults	17	11	64.7	6	1	2	7
Cherambadi .. ..	Children	7	6	85.7	1	1	1	3
	Adults	20	17	85.0	3	1	3	7

\* Horne's spleen size groups were:

I = Umbilicus. II = Four fingers, or handbreadth. III = Two or three fingers.

IV = One finger. V = Not palpable.

† Apparently both adults and children examined.



Viswanathan (*loc. cit.*) reported the following spleen and parasite rates in Gudalur, determined in May 1933 :—

Place.	SPLEEN PALPATIONS.			BLOOD SMEAR EXAMINATIONS.		
	Number examined.	Palpable.		Number examined.	Positive.	
		Number.	Per cent.		Number.	Per cent.
Upper Gudalur ..	58	6	10.3	..	..	..
Middle Gudalur ..	52	9	17.3	..	..	..
Lower { Bandipet ..	15	12	80.0	..	..	..
Gudalur { Chavidipet ..	13	10	76.9	..	..	..
Upper and middle ..	110	15	13.6	15	1	6.7
Lower and Chavidipet ..	28	22	78.6	30	5	16.7

#### COONOR.

It is interesting to note that in 706 examinations in Coonoor in 1940 we found a spleen index of only 0.9 per cent.

In this connection, K. R. Rao (1929a) made a survey in Coonoor in December 1927 and June 1928, and found a spleen rate of 3.5 per cent in 172 examinations of school children. He concluded, '... whereas Coonoor town by itself is for the present practically free from indigenous malaria, the constant and frequently occurring peregrinations of the coolie population to and from some of the low-lying adjoining estates and the plains, which are comparatively well-known endemic foci of the fevers, markedly influence the prevalence of malarial fevers in this municipal area. ... It is only a question of time and meteorological conditions, for both human and mosquito factors to increase in such numbers as to be able to spread effectively the malarial disease, probably even up to endemic proportions.'

Again, K. R. Rao (1930) noted in Coonoor an increasing number of cases of malaria being reported, especially Ottupattarai (our spleen index in 211 examinations in Ottupattarai in 1941, as shown below, was only 1.9 per cent). He pointed to *A. maculatus* as a potential vector and put forward a warning that Coonoor might in time become malarious because of the presence of this species and a growing influx of infected labourers from lower levels. He reported a spleen rate of 2.8 per cent and a parasite rate of 4.8 per cent in Coonoor in 1927-1928.

The annual reports of the Coonoor Public Health Administration since 1936 and probably before, have recorded malaria cases in the Lawley Hospital, listed as 'local' and 'outside' cases as follows:—

Year.	Total malaria cases.	'Local'.	'Outside'.	Residence not classed.
1936	651	258	136	257
1937	547	325	222	..
1938	625	351	274	..
1939	606	308	298	..
1940	657	276	381	..



These data would seem to indicate a serious malaria problem in Coonoor, since ordinarily in South India not more than a tenth of the malarious ill will enter hospital. Accepting these figures, one would have to assume at least 2,500 to 3,500 autochthonous cases of malaria each year in Coonoor with its total population of only 14,326 (1931). Such a degree of endemic malaria would produce spleen indices in children above 30 per cent. Yet in 1940 our spleen index in Coonoor was only 0.9 per cent, and no evidence whatever of malaria transmission was disclosed.

Apart from the ordinary difficulties of getting correct histories from ignorant patients and of diagnosing malaria without using a microscope, it appears that the patients have usually been asked, 'Have you been to the plains?' If the answer is 'no' the patient is classed as a local case. As a matter of fact, the plains around the Nilgiris are usually free from malaria, and the danger of infection is in the hills between the plains and Coonoor. A single night spent only 2,000 feet down the ghat from Coonoor will result in infection in a large proportion of cases. The hospital population of Coonoor has a large proportion of estate labourers who come from lower levels, and it would seem as though the above data have been inaccurately classified.

However, while this report was being prepared, it seemed advisable to make another spleen survey of Coonoor town, including both school and non-school children between the ages of 2 and 14. This was done by courtesy of, and in co-operation with, the Health Officer, Dr. Natarajan. The following were the findings:—

*Spleen survey in Coonoor, April 1941.*

Locality.	Number examined.	PALPABLE.		SPLEEN SIZE.				
		Number.	Per cent.	P.I.	1	2	3	4
Alwarpet School ..	102	0	0.0	..	..	..	..	..
„ Neighbourhood	23	0	0.0	..	..	..	..	..
„ Darlington ..	77	3	..	2	1	..	..	..
Cross Bazaar School ..	219	3	..	1	2	..	..	..
Vinayagarpet ..	180	6	..	3	3	..	..	..
Ottupattarai ..	211	4	..	2	2	..	..	..
„ C. E. Z. M. School.	68	3	..	2	1	..	..	..
Vannarpet ..	272	3	..	1	1	..	1*	..
TOTAL ..	1,152	22	1.9	11	10	..	1	..

\*This girl had arrived in Coonoor two weeks prior to examination. She came from Bengal. Both she and her father were suffering from chronic malaria (*P. vivax* infection).



At the time we made this extra spleen survey in Coonoor, we also collected anophelines, but once again failed to find *A. fluviatilis*.

We feel justified in stating that we found no evidence at all of malaria transmission in 1940 or 1941 in Coonoor. We do not believe that malaria was even hypo-endemic within the municipal limits. However, we cannot rule out the possibility that in some previous and unusual year there might have been some transmission in this area.

#### WELLINGTON, ARUVANKADU AND KETTI.

In October and November 1941, after this paper had been completed, we were requested to make spleen surveys in Wellington, Aruvankadu and Ketti, where the elevations are 6,000, 6,300, and 6,600 feet respectively. The following data were obtained:—

#### *Spleen survey, children in Wellington, Aruvankadu and Ketti.*

Group.	Number examined.	PALPABLE.		SPLEEN SIZE.					
		Number.	Per cent.	P.I.	1	2	3	4	
<i>Wellington—</i>									
Europeans resident 1 year or more	47	3	6.4	2	1	..	..	..	
Europeans recently arrived ..	29	4	13.8	2	2	..	..	..	
Indian camp-followers resident 1 year or more.	41	3	7.3	1	..	2	..	..	
Indian camp-followers recently arrived.	51	29	56.9	9	10	10	2	..	
St. Joseph's School resident 1 year or more.	258	12	4.7	8	4	..	..	..	
<i>Aruvankadu—</i>									
Cordite Factory School resident 1 year or more.	261	8	3.1	7	1	..	..	..	
<i>Ketti—</i>									
St. George's Homes resident 1 year or more.	173	7	4.0	4	3	..	..	..	

In the above table, it will be noted that at Wellington some of the children of European military personnel and of Indian camp-followers had recently arrived. They came from Sialkot in Northern India and had been in Wellington less than a month. Among the four recently arrived European children with palpable spleens there was one with a positive smear (*P. falciparum*). Among the 29 Indian children with palpable spleens there were 20 with positive blood smears. Of these, 11 were *P. vivax* and 10 were *P. falciparum*, one smear containing both species.

In the St. George's Homes at Ketti in September 1941, there had been a unique outbreak of malaria, a disease never before observed in these Homes.



Among the 173 children there were seven cases, all in older children, all with positive blood smears (*P. vivax* 3, *P. falciparum* 5, one smear with both species).

The following anophelines were collected at Ketti in October 1941.

*Anophelines collected at Ketti in October 1941.*

Species.	Larvæ.	Adults.
<i>A. aitkeni</i> ..	43	1
<i>A. culicifacies</i> ..	..	1
<i>A. gigas</i> ..	19	3
<i>A. jeyporiensis</i> ..	9	1
<i>A. maculatus</i> ..	27	..
<i>A. splendidus</i> ..	3	1

All of the adults were dissected and found negative.

These findings in Wellington, Aruvankadu and Ketti are discussed below.

OOTACAMUND.

We made no spleen examinations in Ootacamund, where there appears to be no malaria. V. S. Rao (1916) reported 1,527 examinations of children with a spleen index of 2.5 per cent. Omitting children having a history of residence in lower malarious places, his index was 1.4 per cent. K. R. Rao (1929b) examined 502 children and found a spleen rate of 0.2 per cent. He concluded: 'There is no malaria in Ootacamund, nor are there any possibilities of its incidence in the near future, inasmuch as the malaria-carrying mosquitoes are not known to be present'.

TABLE XIX.

*Spleen palpations of adults, Nilgiris east.*

Place.	Date, 1940.	Number examined.	PALPABLE.	
			Number.	Per cent.
Burliar .. ..	Apr. 27	58	15	25.6
Burliar .. ..	Oct. 13	43	7	16.3
Kallar .. ..	Oct. 27	30	4	13.0
P. W. D. road coolies on Coonoor-Mettupalayam road.	May 16	36	6	16.7
TOTAL .. ..	..	167	32	19.2



TABLE XX.

*Spleen palpations grouped according to altitude of places visited.*

Places included.	Altitude (feet).	SPLEENS.		Notes.
		Number examined.	Per cent palpable.	
Dayanur, Mettupalayam, Pungampalayam, Tekkampatti, Tholampalayam, Vellingodu (all places in this group located in flat areas at least 4 miles from foothills).	1,000 to 1,100	479	4.4	No <i>A. fluviatilis</i> found at this distance from foothills.
Kallar and Nellithurai ..	1,200 to 1,999	114	78.1	<i>A. fluviatilis</i> prevalent at these altitudes (see Table X).
Burliar, Benhope, Devarshola, Devala, Gudalur, Massinigudi, Nellakkotta, Singara, Tepakkadu.	2,000 to 3,999	570	50.4	
Coonoor, Devarshola, Jekkanneri*, Kilinjada*, Kilkotagiri, Kilkunda, Kodanad, Kolakambi*, Konakkarai, Melkunda, Naduvattam*, Nirkundi, Nonsuch Estate, Prospect Estate, Selas, Sholurmattam.	4,000 or higher.	1,562	6.0	No <i>A. fluviatilis</i> found at this altitude.

\* The spleen rates at these places are 17.4, 37.5, 23.4 and 12.9 per cent respectively, chiefly due to the presence of estate coolies who were probably infected at lower levels.

In Table XIX are listed records of some spleen palpations in adults in the Nilgiris east area. In Table XX, spleen palpations are grouped by altitude of place where examinations were made. It will be noted that between 1,000 and 1,100 feet the rate was 4.4 per cent, from 1,200 to 1,999 feet it was 78.1 per cent, from 2,000 to 3,999 feet it was 50.4 per cent, and above 4,000 feet it was only 6.0 per cent. The rates for the first and last divisions might have been lower, could we have excluded labourers probably infected in the other two zones. This table taken with Table X, which shows that the vector *A. fluviatilis* was not found above 4,000 feet, would seem to indicate that areas above 4,000 feet are relatively not malarious. There may no doubt be exceptions to this general rule, and, as already noted, it is possible that in some years, *A. fluviatilis* may occur at levels as high as 5,500 feet. This may be true in situations like that at Nonsuch Estate, where labour lines are situated at about 5,000 feet near the face of a cliff dropping sharply to a stream which is below 4,000 feet. Another exception is Terramia Estate, near Kolakambe, where the average temperatures at 5,000 feet are those normal for 4,000 feet elsewhere, owing to the configuration of the valley, which appears to 'trap' the heat.



TABLE XXI.

Blood smear examinations, Nilgiris area, 1940.

Place.	Date.	Number examined.	Positive.		SPECIES*.			
			Num-ber.	Per cent.	F.	V.	Mal.	Mxd.
Devala .. ..	May 24	74	30	40.1	10	12	8	0
Gudalur .. ..	Feb. 16	69	19	27.5	13	6	0	0
Gudalur .. ..	Aug. 29	104	32	30.7	8	8	16	0
Massinigudi ..	Aug. 29	26	10	38.5	7	3	0	0
Nilgiris west total ..	=	273	91	33.3	38	29	24	0
Benhope .. ..	May 15	27	15	55.6	5	5	5	0
Burliar .. ..	Apr. 12	94	42	44.7	5	1	37	1†
Burliar .. ..	Oct. 13	80	32	40.0	4	1	27	0
Kallar .. ..	Mar. 27	60	32	53.3	4	2	26	0
Kallar .. ..	June 1	24	12	50.0	4	0	8	0
Kallar .. ..	Oct. 27	50	23	46.0	3	3	17	0
P. W. D. road coolies	May 16	36	13	36.1	0	4	9	0
Nilgiris east total ..	=	371	169	45.6	25	16	129	1†
Coonoor .. ..	March and April.	706	2	0.3	1	1	0	0
Kolakambi‡ ..	Feb. 12	77	10	13.0	9	0	1	0
Nonsuch Estate ..	May 26	101	2	1.9	0	2	0	0
Total including Nilgiris west and east.	=	884	14	1.6	10	3	1	0

\* F.=*P. falciparum*; V.=*P. vivax*; Mal.=*P. malariae*; Mxd.=Mixed infection.† Mixed *falciparum* and *malariae* infection.

‡ Estate coolies, probably infected at lower levels.

Blood smear examinations are listed in Table XXI. Here again there is evidence that the Devala-Gudalur and Kallar areas are malarious but that Coonoor is not. It is of interest to note the prevalence of *P. malariae* in the Nilgiris, especially in Nilgiris east, where it was the commonest species.

Infant malaria indices for the Devala-Gudalur area are given in Table XXII. These are very high, ranging from 40.0 to 59.8 per cent. They indicate a serious degree of malaria transmission. They may be contrasted with indices



TABLE XXII.

*Infant malaria indices in Gudalur and Devala, Nilgiris west.\**

Place.	Date.	Number examined.	POSITIVE SMEARS.		SPECIES†.			
			Num-ber.	Per cent.	F	V.	Mal.	Mxd.
Devala area ..	Nov. 1-15, 1940	85	46	54.1	9	25	12	0
Devala area ..	Jan. 17-22, 1941	82	49	59.8	37	7	7	2‡
Gudalur area ..	Nov. 1-15, 1940	25	10	40.0	5	3	3	1‡
Gudalur area ...	Jan. 17-22, 1941	36	17	47.2	4	9	6	2‡
TOTAL ..		228	122	53.5	55	44	28	5‡

\* Only infants one year of age or less included.

† F. = *P. falciparum*; V. = *P. vivax*; Mal. = *P. malariae*; Mxd. = Mixed infection.‡ Mixed *falciparum* and *malariae* infection.

up to 17.1 per cent in Mysore and up to 10.6 per cent in Pattukkottai Taluk, reported by Russell, Sweet and Menon (1939).

TABLE XXIII.

*Infant malaria survey. Data analysed by months of birth and of exposure.*

ANALYSIS BY MONTHS OF BIRTH.				ANALYSIS BY MONTHS OF EXPOSURE*.		
Months.	Number examined.	Positive.		Number examined	Positive.	
		Number.	Per cent.		Number.	Per cent.
January ..	18	14	77.8	131	100	76.3
February ..	24	17	70.8	67	51	76.1
March ..	17	10	58.8	84	61	72.6
April ..	21	13	61.9	105	74	70.5
May ..	17	9	52.9	123	83	67.5
June ..	10	2	20.0	132	85	64.4
July ..	23	14	60.9	155	99	63.9
August ..	16	3	18.8	171	102	59.7
September*	20	9	45.0	191	111	58.1
October ..	22	4	18.2	213	115	54.0
November ..	31	21	67.7	134	80	59.7
December ..	9	6	67.7	143	86	60.1

Notes.—\* Each infant was assumed to have been exposed to infection each month from birth to date of examination. If an infant was found positive, it was further assumed that the infection might have occurred in any month of exposure.

Survey dates November 1-15, 1940, and January 17-22, 1941.



In Table XXIII the infant malaria data are analysed by month of birth of infant and by months of exposure. In the latter case, each infant was assumed to have been exposed to infection each month from birth to date of examination.

TABLE XXIV.

*Positive blood smears found in individuals having various degrees of splenic enlargement, Nilgiris area, 1940.*

Spleen size group.	Number in group.	WITH POSITIVE BLOOD SMEARS.	
		Number.	Per cent.
Not palpable ..	415	92	22.2
Palpable on inspiration	35	9	25.7
I .. ..	107	63	58.8
II .. ..	96	61	63.5
III .. ..	36	25	69.4
IV .. ..	27	18	66.7
TOTAL ..	716	268	37.4

TABLE XXV.

*Positive blood smears found in individuals having various degrees of splenic enlargement. Three areas compared and totalled.*

Place.	PERCENTAGE OF POSITIVE SMEARS IN VARIOUS SPLEEN SIZE GROUPS.					
	Negative.	P.I.	I.	II.	III.	IV.
Pattukkottai Taluk(1)	4.8	50.3	82.6	90.1	92.3	41.3
Ennore-Nellore coastal area(2).	34.2	56.5	87.0	89.2	94.0	85.7
Nilgiris(3) ..	22.2	25.7	58.8	63.5	69.4	66.7
Total examined . ..	3,597	851	772	509	249	115
Total positive ..	334	424	617	432	223	78
Per cent positive ..	9.3	49.8	79.9	84.7	89.6	63.5

Note.—(1) See Russell, Menon and Rao (1938), Table IX.

(2) See Russell and Jacobb (1939), Table XIV.

(3) See Table XXIII above.



If an infant was found positive, it was further assumed that the infection might have occurred in any month of exposure. Only infants of a year or less in age were included in these surveys. Analysis by month of exposure in this way was suggested by Russell, Menon and Rao (*loc. cit.*) and developed by Russell, Sweet and Menon (*loc. cit.*). It would appear from this analysis that malaria transmission in Nilgiris west occurs throughout the year, but is least active from August to November.

In Table XXIV the various degrees of splenic enlargement are analysed in respect of positive blood smears. It is of interest to note that 25.7 per cent of the P.I. spleens occurred in children having a positive blood smear. Similar results have been noted in other areas, as shown in Table XXV. In a total of 3,597 published spleen examinations, by the authors or their colleagues, there were 851 spleens classed as P.I. Among these, 49.8 per cent were associated with positive blood smears. This may be contrasted with a rate of 9.3 per cent for spleens not palpable even on deep inspiration. In the Nilgiris area, as elsewhere, there have been fewer positive smears associated with class IV than with class III spleens.

TABLE XXVI.

*Splenic enlargement in relation to species of Plasmodium.*

Plasmodium species.	Total positive smears.	PERCENTAGE OF TOTAL POSITIVES FOUND IN THESE SPLEEN SIZE GROUPS.						All palpable.
		Not palpable.	P.I.	1	2	3	4	
<i>P. falciparum</i> ..	73	32.9	5.5	27.4	23.3	4.1	6.8	69.1
<i>P. vivax</i> ..	48	50.0	4.2	20.8	22.9	0.0	2.1	50.0
<i>P. malariae</i> ..	154	31.8	1.9	21.4	22.7	14.3	7.8	68.2

Note.—Percentages of positive smears in the combined size 3 and 4 groups.

*P. vivax* .. 2.1 per cent.

*P. falciparum* .. 10.9 per cent.

*P. malariae* .. 22.1 per cent.

TABLE XXVII.

*Hæmoglobin determinations.*

Place.	Date, 1940.	Number examined.	HÆMOGLOBIN.	
			Per cent average.	Grams per 100 c.c. of blood average.
Burliar ..	Oct. 13	80	61	9.8
Gudalur ..	Dec. 13	126	79	12.8
Kallar ..	Oct. 27	50	51	8.4

Note.—New improved Dare hæmoglobinometer used for these determinations.



Table XXVI shows data regarding splenic enlargement in relation to species of *Plasmodium*. It will be noted that 50.0 per cent of children having *P. vivax* in blood smears did not have a palpable spleen. The figures for *P. falciparum* and *P. malariae* were 32.9 and 31.8 per cent, respectively. As regards the largest spleens, 22.1 per cent of the *P. malariae* positive children had spleens of class III or class IV. The figures for *P. vivax* and *P. falciparum* were 2.1 and 10.9 per cent, respectively.

In Table XXVII are given results of some hæmoglobin determinations, for which a new improved Dare hæmoglobinometer was used. As might be expected, the percentages are low.

### DISCUSSION.

The foregoing report, regarding the epidemiology of malaria in the Nilgiris, confirms an old local belief that the ghat areas are malarious but the central plateau usually is not. We might quote, for instance, Baikie (1857, p. 125) who wrote, 'Persons who have suffered from fever, should be cautious to avoid passing through the jungle at the foot of the hills during the night; and, if unluckily detained in there after sun-set, they should on their arrival submit to a course of purgatives, followed by quinine in small doses.'

Another quotation is from Grigg (1880) who reported, 'The danger of visiting the belts of jungle which surround and clothe the base of the Nilgiris to a height of from 2,000 to 3,500 feet, was exemplified in the early month of the year 1876. A party of 35 constables proceeded to arrest certain criminals who had taken rest there. On their return, after remaining for about two months in this unhealthy locality, all without exception suffered from malarious fever, so much so that two died shortly after their return to Ootacamund, seventeen had to proceed on sick leave, and of the remainder several, after a lapse of several months, occasionally complained of the return of the fever or other attendant ills.'

### INTRODUCED MALARIA.

Although it seems clear from the foregoing report that malaria is not usually endemic in the central plateau of the Nilgiris, yet obviously from the Ketti outbreak mentioned above, and from the observations of local physicians, one must expect occasional transmission above the fairly distinct boundary at 4,000 feet elevation.

The Ketti outbreak had some interesting features. It occurred only among older children who had for some weeks been practising for annual games. The playing field is located beside a stream in a valley some 500 feet below the school and this valley descends directly to the plains. These older children had been remaining at the field until after dark. There were seven undoubted cases of malaria and although in three of these relapses might have been considered, since the children came originally from malarious places, in the other four cases the children had been in Ketti for several years and had come from normally non-malarious places. Very careful questioning made it practically certain that only one of these children had been down the ghats below 6,000 feet within a year and this one child only in daytime. It seems beyond doubt that these children actually contracted malaria at Ketti, probably at an elevation of about 6,100 feet.



Painstaking collections, scanty because of the rains, failed to reveal any *A. fluviatilis*, but one *A. culicifacies* adult was taken. The latter species, in our experience, is rare at this altitude. It is a known carrier on the plains wherever it has sufficient density. *A. aitkeni*, *A. gigas* and *A. maculatus* were fairly abundant. None has been found to be a carrier in the Nilgiris. There had been unusual rains in September and the density of *Anopheles* had been higher than usual.

Perhaps the most important point about the Ketti outbreak is the fact that during 1941 more than two thousand labourers have been imported to nearby Aruvankadu, many from malarious areas. There must certainly be many gametocyte carriers among these labourers, and it seems reasonable to suppose that the local anophelines may under such circumstances occasionally transmit malaria. The Medical Officer at Aruvankadu had observed in November 1941 two cases of malaria in adults who had not been out of this village for some years. Although the school children at Aruvankadu have not yet shown evidence of transmission, it would not be surprising if they did so during 1942.

At Wellington Cantonment (6,000 feet elevation) there was in November one case which appeared certainly to have been contracted locally. Here, too, many gametocyte carriers had recently been imported. For example, we found a spleen rate of 56.9 per cent among newly arrived children of Indian camp-followers who had come from Sialkot, in Northern India. Among the 29 children with palpable spleens there were 20 with positive smears and of these, 16 showed gametocytes. Doubtless, many of the parents were also gametocyte carriers. Here, then, is a focus of 'immigrant', or imported, malaria which might be great enough to permit some local anophelines temporarily to transmit malaria.

Three facts make the Nilgiris plateau liable to occasional transmission of malaria:—

- (a) This plateau is surrounded by hyperendemic malaria on all the ghats from 1,200 to 4,000 feet elevation. There are numerous tea, coffee, and cardamom plantations in this hyperendemic zone, and infected labourers naturally gravitate to the plateau where the chief cities of the Nilgiris are found.
- (b) The plateau is not only a health resort to which visitors come from malarious areas, but it is also the location of a military cantonment and an important military factory, to both of which large numbers of gametocyte-carrying individuals may come.
- (c) There is a rich anopheline fauna on the plateau, and although no plateau species has yet been proved to be a vector, possibly when the number of imported gametocyte carriers is large, the small percentage of local anophelines which fulfil the conditions required for successful transmission of malaria may be large enough to produce sporadic cases. These cases would be autochthonous, but introduced rather than indigenous (League of Nations, 1940).

#### SIR RONALD ROSS.

We feel justified in slightly prolonging this discussion by calling attention to an almost forgotten visit that Ross (1898) made to Sigur Ghat, near Ootacamund, in April, May and June 1897, before his epochal discovery in



Secunderabad in August 1897, and his later proof, in Calcutta, of the bird-mosquito-bird chain of malaria infection. In his report of his Nilgiris visit Ross postulated an external form of the malaria parasite which he called the 'x-protozoön'. This he said could be (a) 'free in air, water, soil, rotting vegetation, etc., and capable of an optional parasitism in man'; (b) 'a parasite in another animal, by which it is communicated directly or indirectly to man'; (c) both (a) and (b).

Ross went to Sigur Ghat to investigate the second hypothesis because 'certain recent inductions' of Manson, Bignami and himself strongly suggested 'that the mosquito harbours the x-protozoön'. He, therefore, proposed to study in Sigur Ghat the following questions:—

- (1) 'Are mosquitoes sufficiently numerous in very malarious localities to account for the extreme prevalence of the disease there?'
- (2) 'Do mosquitoes in such localities harbour a parasite morphologically similar to the *hæmamoeba* in man?'

Although he found no 'mosquito grubs' in the pools of the ghat stream, he did find numerous voracious jungle mosquitoes (*Aedes*?) and answered his first question in the affirmative, although he thought that 'apart from Manson's induction', hypothesis (a) was 'just as probable, if not more so, than the mosquito theory'. He failed to find any parasites in these mosquitoes 'very similar to the *hæmamoeba*', and thus Sigur Ghat missed the immortality conferred a little later, on Secunderabad.

Ross stated that malaria extended up the ghat to Kalhutti, at an elevation of 5,500 feet, three miles from Ootacamund. But his evidence did not rule out the possibilities that the Kalhutti infections had originated lower down the ghat.

### SUMMARY AND CONCLUSIONS.

1. The amount and seasonal distribution of rainfall in the Nilgiris are markedly different in the east from those in the west. This factor in turn influences the seasonal prevalence of such stream-breeding anophelines as *A. fluviatilis*.

2. The anopheline fauna of the Nilgiris is very large, containing no less than 23 species, the larval habitats of which have been discussed.

3. Altitude appears to be to some extent a factor in determining distribution of anopheline species. *A. aitheni* and *A. gigas* are most abundant above 4,000 feet, *A. fluviatilis* below 4,000 feet and above 1,000 feet, *A. culicifacies* and *A. subpictus* below 2,000 feet.

4. *A. fluviatilis* is the chief and probably the only malaria vector in the Nilgiris. It is prevalent throughout the year in Nilgiris east, most abundant from April to July. In the west, larvæ are most abundant from February to June. During the south-west monsoon, July to October, these larvæ are relatively scarce.

5. The infection index in *A. fluviatilis* was 17.3 per cent in 2,580 dissections. Weekly sporozoite indices as high as 34.8 per cent were found in Nilgiris east (Kallar).

6. Spleen rates varied from 100 per cent in Kallar (Nilgiris east) and 51.9 per cent in Gudalur (Nilgiris west) to 0.9 per cent in Coonoor (Nilgiris west).



central). The average for Nilgiris east was 84.5 and for Nilgiris west, 46.5 per cent.

7. Spleen rates varied with altitudes as follows: 1,000 to 1,100 feet, 4.4 per cent; 1,200 to 1,999 feet, 78.1 per cent; 2,000 to 3,999 feet, 50.4 per cent; 4,000 feet or higher 6.0 per cent.

8. Parasite rates varied from 53.3 per cent in Kallar and 30.7 per cent in Gudalur to 0.3 per cent in Coonoor. The average for Nilgiris east was 45.6 per cent and for Nilgiris west 33.3 per cent. *P. malariae* is the most common species in Nilgiris east, but is relatively less common in Nilgiris west.

9. Infant malaria indices were high, ranging in the Devala-Gudalur area, of Nilgiris west, from 40.0 to 59.8 per cent. They indicated that transmission probably occurs throughout the year but is least active from August to November.

10. *P. malariae* produced the greatest percentage of large spleens (classes III and IV) and *P. vivax* the smallest percentage. Half of the children with *P. vivax* infections did not have an enlarged spleen at the time of examination.

11. It appears that, while the eastern and western ghats of the Nilgiris are highly malarious, the central plateau is not, the dividing line coming at about 4,000 feet elevation. This line may perhaps rise to 5,500 feet in exceptional years or places.

12. Although the central Nilgiris plateau is usually not subject to autochthonous malaria, there may be sporadic transmission following an unusually large influx of imported malaria. Such sporadic cases would be autochthonous, but of the *introduced* rather than *indigenous* variety.

## REFERENCES.

- |   |    |    |   |
|---|----|----|---|
| BAIKIE, R. (1857)                       | .. | .. | <i>The Nilgherries</i> . 2nd ed. Calcutta: Baptist Mission Press.   |
| CHALAM, B. S. (1923)                    | .. | .. | Some notes on a malaria investigation on a sugar estate in Kamrup, Assam. <i>Ind. Med. Gaz.</i> , <b>58</b> , 10, pp. 476-479.  |
| COVELL, G. (1927)                       | .. | .. | A critical review of the data recorded regarding the transmission of malaria by the different species of Anopheles with notes on distribution, habits and breeding places. <i>Ind. Med. Res. Mem.</i> No. <b>7</b> , pp. 46-47. |
| <i>Idem</i> (1931)                      | .. | .. | The present state of our knowledge regarding the transmission of malaria by the different species of anopheline mosquitoes. <i>Rec. Mal. Surv. Ind.</i> , <b>2</b> , 1, pp. 1-48.   |
| COVELL, G., and HARRHAGWAN (1939).      |    |    | Malaria in the Wynad, South India. <i>J. Mal. Inst. Ind.</i> , <b>2</b> , 4, pp. 341-376.   |
| COVELL, G., and PURI, I. M. (1936).     |    |    | The distribution of anopheline mosquitoes in India. <i>Health Bulletin</i> 17. Manager of Publications, Delhi.  |
| GRIGG, H. B. (1880)                     | .. | .. | Manual of the Nilgiri District. (Publisher ?) (Copy in Coonoor Library, Vol. R/1164.)   |
| HOLT, R. L., and RUSSELL, P. F. (1932). |    |    | Malaria and Anopheles reconnaissance in the Philippines. <i>Philipp. J. Sci.</i> , <b>49</b> , 3, pp. 305-371.  |
| HORNE, J. H. (1913)                     | .. | .. | Report on the malaria investigation in Wynad and its vicinity. (This report was printed by the Madras Govt. as pp. 13-20 of a file of papers dated 15th April, 1913.)   |



- HORNE, J. H. (1914) .. Malaria in Wynaad. *Proc. 3rd All-India San. Conf. Lucknow (Suppt. to Ind. J. Med. Res., 4, pp. 71-74).*
- KING, H. H., and IYER, M. K. R. (1929). Second report on malaria in Mopad (Nov. 1928). *Suppt. to Ann. Rep. King Inst., Guindy, 1927-28, pp. 10-15.*
- LEAGUE OF NATIONS (1940) .. Report on terminology in Malaria. *Bull. Hlth. Org. L. of N., 9, 2, pp. 158-159.*
- MATHEW, M. I. (1939) .. Anopheline transmitters of malaria in South Travancore. *J. Mal. Inst. Ind., 2, 1, pp. 101-104.*
- MEASHAM, J. E. (1939) .. *The transmission and prevention of malaria on the tea estates of the Madras Presidency.* 56 pp. Valparai.
- PERRY, E. L. (1914) .. Endemic malaria of the Jeypore Hill Tracts of the Madras Presidency. *Ind. J. Med. Res., 2, 2, pp. 456-491.*
- RAO, K. R. (1929a) .. Report on malaria survey in Coonoor Municipality. Madras : Supdt., Govt. Press.
- Idem (1929b) .. Report on malaria survey in Ootacamund Municipality. Madras : Supdt., Govt. Press.
- Idem (1930) .. Note on the present state of knowledge about malaria in the Presidency of Madras. *Rec. Mal. Surv. Ind., 1, 4, pp. 473-497.*
- RAO, V. S. (1916) .. Report on prevalence of malaria in Ootacamund. Govt. of Madras. Local and Municipal Dept., G.O. No. 1538 M., August 30.
- ROSS, R. (1898) .. Report on a preliminary investigation into malaria in Sigur Ghat, Ootacamund. *Ind. Med. Gaz., 33, 4, pp. 133-136; 33, 5, pp. 173-175.*
- RUSSELL, P. F., and JACOB, V. P. (1939). Epidemiology of malaria in the Ennore-Nellore coastal area, Madras Presidency, India. *J. Mal. Inst. Ind., 2, 2, pp. 131-152.*
- RUSSELL, P. F., MENON, M. K., and RAO, T. R. (1938). Epidemiology of malaria in Pattukkottai Taluk. Tanjore District, Madras Presidency, India. *Ibid., 1, 3, pp. 285-326.*
- RUSSELL, P. F., and RAO, T. R. (1940). Natural malaria infections in some South Indian anophelines, with special reference to *A. culicifacies*. *Ibid., 3, 4, pp. 543-562.*
- Idem (1941) On seasonal prevalence of Anopheles species in South-eastern Madras. *Ibid., 4, 2, pp. 263-296.*
- RUSSELL, P. F., SWEET, W. C., and MENON, M. K. (1939). Some observations on malaria parasite rates in infants in South India. *Ibid., 2, 4, pp. 439-455.*
- VISWANATHAN, D. K. (1936) .. Report on the anti-malarial operations carried out from the Government of India grant in the Madras Presidency. 29 pp. Madras : Supdt., Govt. Press.



MALARIA CONTROL BY SPRAY-KILLING ADULT  
ANOPHELES, SECOND SEASON'S RESULTS :  
WITH SPECIAL REFERENCE TO THE  
EFFECTS OF THIS MEASURE ON THE  
LONGEVITY AND INFECTIVITY OF  
*ANOPHELES MINIMUS*.

BY

D. K. VISWANATHAN, L.M.S., B.S.SC., M.P.H. (Johns Hopkins),  
(Research Officer, Assam Medical Research Society, Shillong).

[January 15, 1942.]

INTRODUCTION.

IN a previous paper (Viswanathan, 1941), the results of an experiment in malaria control in a hyperendemic tea garden in Upper Assam by the use of pyrocide 20 as an insecticidal spray were described. During 1940, a portion of the coolie lines was sprayed once weekly, the remainder being left unsprayed for comparative purposes. As a result of the spraying, there was a marked diminution in transmission in the sprayed area, as evidenced by a reduction in the parasite index and infant malaria index, as well as in the infection index of *A. minimus*. The hospital admissions diagnosed as malaria also showed a considerable diminution. There was, however, no demonstrable reduction in the output of *A. minimus* larvæ, and only a very slight reduction in the density of adult infestation with this species. When spraying is carried out once weekly, *A. minimus* apparently lives long enough to breed, but not long enough to transmit.

During 1941, all the lines were sprayed once weekly from April 15 to December 10, whilst some of the lines were sprayed twice weekly from June 1 to October 31. In addition to the routine collection of such data as spleen rates, parasite indices, infant indices, infection indices and malaria morbidity figures, the age of adult specimens of *A. minimus* collected from lines sprayed once a week and twice a week and from unsprayed lines in an adjoining tea garden, was determined on three occasions, as indicated by the wing grade, ovarian development and gut contents of the specimens.



## SECOND SEASON'S RESULTS.

## 1. MALARIA INCIDENCE.

Table I shows the annual malaria admissions to hospital from 1933 to 1941, the percentage of malaria to all cases admitted and the malaria incidence per mille of population.

TABLE I.  
*Limbuguri Tea Estate.*  
*Hospital figures for malaria incidence, 1933-1941.*

	1933.	1934.	1935.	1936.	1937.	1938.	1939.	1940.	1941.
Malaria cases .. ..	4,202	4,151	3,127	1,741	2,353	1,726	1,675	881	771
Percentage of malaria to all cases admitted.	34	33	33	19	26	21	20	13	11
Malaria incidence per mille of population.	1,666	1,626	1,131	632	900	658	621	316	278

The great reduction in the incidence of malaria which occurred in 1940 as a result of spraying once weekly in half the coolie lines was thus maintained, and even slightly bettered, in 1941. But during this year, all the coolie lines were sprayed once weekly and some lines twice weekly. In all the adjoining gardens the incidence of malaria in 1941 was considerably higher than in 1940 due, principally, to the early outbreak of the monsoon in April and to some spells of dry weather later in the year.

*Seasonal incidence.*—Table II and Graph 1 show the seasonal incidence of malaria in 1940 and 1941 respectively, as compared with the average incidence from 1933 to 1939.

TABLE II.  
*Limbuguri Tea Estate.*  
*Seasonal malaria prevalence for 1933 to 1939, 1940 and 1941.*

Period.	Jan.	Feb.	Mar.	April.	May.	June.	July.	Aug.	Sept.	Oct.	Nov.	Dec.
1933 to 1939 average.	104	112	97	114	162	299	366	406	389	290	247	141
1940	70	62	47	51	50	90	105	103	87	92	71	50
1941	48	27	35	36	32	56	108	95	174	60	57	46

Except in the month of September, the malaria prevalence was less in every month in 1941 than in 1940, and much less than the average incidence in 1933 to 1939. Malaria morbidity seldom exhibits such a sharp fastigium as was shown in September 1941. The peak is generally of the nature of a truncated cone. As there was an epidemic of influenza during September, some of these cases were probably diagnosed as malaria.



GRAPH 1.

## LIMBUGURI TEA ESTATE.

Seasonal malaria prevalence for 1933 to 1939, 1940 and 1941.

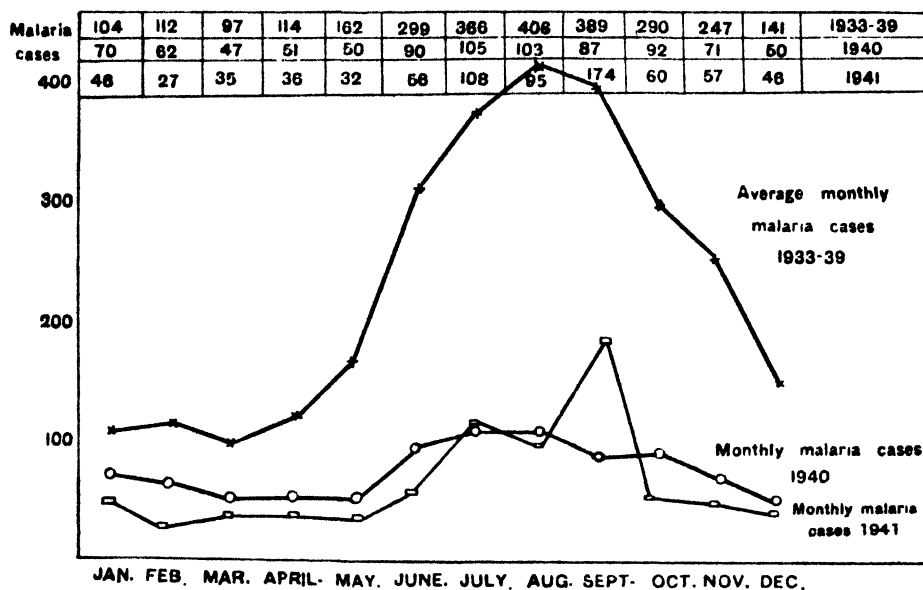


Table III shows the number of malaria cases in the various groups of lines subjected to different kinds of experiment in 1940 and 1941 respectively.

TABLE III.

## Limbuguri Tea Estate.

Malaria incidence by coolie lines, 1940 and 1941.

Coolie lines.	MALARIA CASES UP TO THE END OF NOVEMBER.			$\frac{(T-O)^2}{T}$
	1940.	1941.		
		Observed (O).	Expected (T).	
Unsprayed in 1940: sprayed once weekly in 1941.	255	283	230	12.2
Sprayed once weekly in 1940 and 1941 ..	40	48	35	4.8
Unsprayed in 1940: sprayed twice weekly in 1941.	139	109	119	0.9
Sprayed once weekly in 1940: twice weekly in 1941.	444	351	407	7.7
TOTAL ..	878	791	..	25.6



The observed distribution of malaria cases in 1941 in the various lines is, therefore, significantly different from the expected distribution had the lines been subjected to the same kind of experiment. The variations in the main are:—

(1) an increase in incidence in 1941 in the lines sprayed once weekly in both years, indicating a higher degree of transmission during that year;

(2) a much higher increase in 1941 in lines not sprayed in 1940 but sprayed once weekly in 1941, suggesting that the absence of spraying in 1940 relatively loaded the human reservoir factor and increased the hazard of transmission;

(3) a decline in incidence in 1941 in lines not sprayed in 1940 but sprayed twice weekly in 1941, showing that despite the same degree of loaded human reservoir factor in these lines as in (2) and despite increased transmission in 1941, spraying twice weekly reduced the malaria incidence; and

(4) a much larger reduction in incidence in 1941 in lines sprayed once weekly in 1940 and twice weekly in 1941, showing the combined effects of a reduction in the human reservoir due to the previous year's spraying and a diminution in transmission due to spraying twice weekly in 1941.

## 2. SPLEEN RATES AND AVERAGE ENLARGED SPLEEN.

The spleen rates show no reduction even after two years of spraying. They were 80 per cent before the commencement of spraying operations in 1940 and they are 79 per cent in December 1941. But the average enlarged spleen which was 9 cm. (apex to umbilicus) and 7 cm. (apex to midline) in April 1940, has been reduced to 10 cm. and 8 cm. respectively.

## 3. PARASITE INDICES.

Table IV shows that there was a steady decline in the parasite indices in children aged from 2 to 10 years during the period January 1940 to December 1941.

TABLE IV.

*Limbuguri Tea Estate.*

*Parasite indices among children aged 2 to 10 years, 1940 and 1941.*

	1940.			1941.		
	January.	June.	December.	April.	August.	November.
Parasite index ..	48.4	41.4	35.8	30.4	27.8	24.2

## 4. INFANT MALARIA INDEX.

This was 31.6 per cent in December 1940 and 13.1 per cent in December 1941. This is the most sensitive index of the extent of malaria transmission. It will be seen that the spraying of all the lines in 1941 (twice weekly during the active malaria season) has considerably reduced transmission.

## 5. DENSITY OF LARVAL BREEDING.

Table V and Graph 2 show the density of *A. minimus* larvæ (reduced to 10 man-hours per month) in 1940 and 1941 respectively.



## GRAPH 2.

## LIMBUGURI TEA ESTATE.

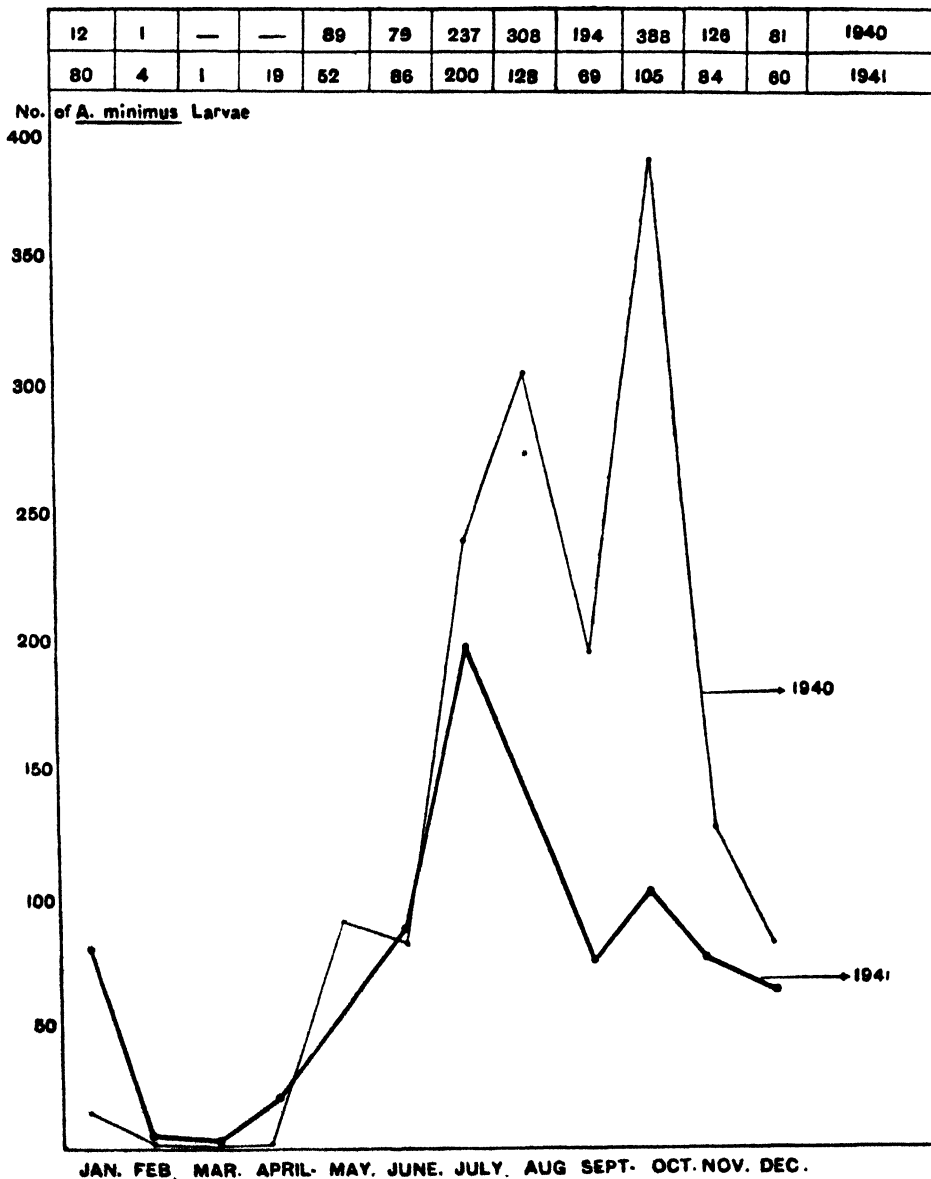
Seasonal prevalence of *A. minimus* larvæ in 1940 and 1941.



TABLE V.

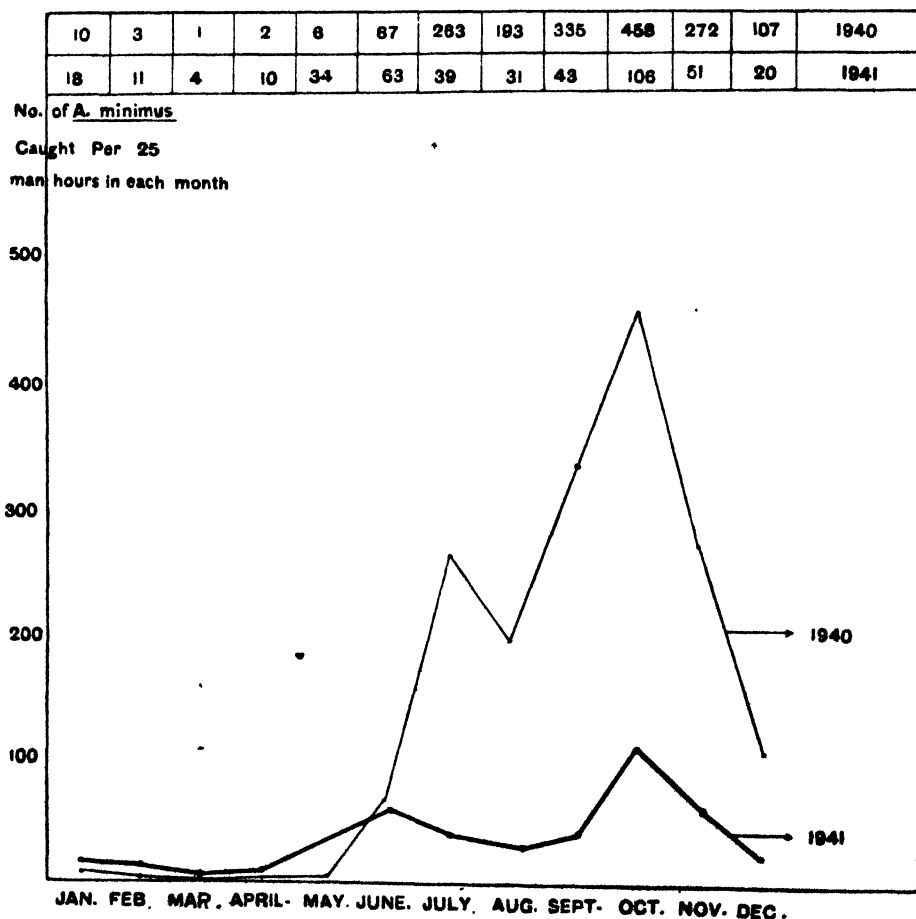
Limbuguri Tea Estate.

*A. minimus* larva—seasonal prevalence, 1940 and 1941.

Year.	Jan.	Feb.	Mar.	April.	May.	June.	July.	Aug.	Sept.	Oct.	Nov.	Dec.
1940	12	1	..	..	89	79	237	308	194	388	126	81
1941	80	4	1	19	52	86	200	128	69	105	84	60

GRAPH 3.

LIMBUGURI TEA ESTATE.

Seasonal prevalence of *A. minimus* in 1940 and 1941.

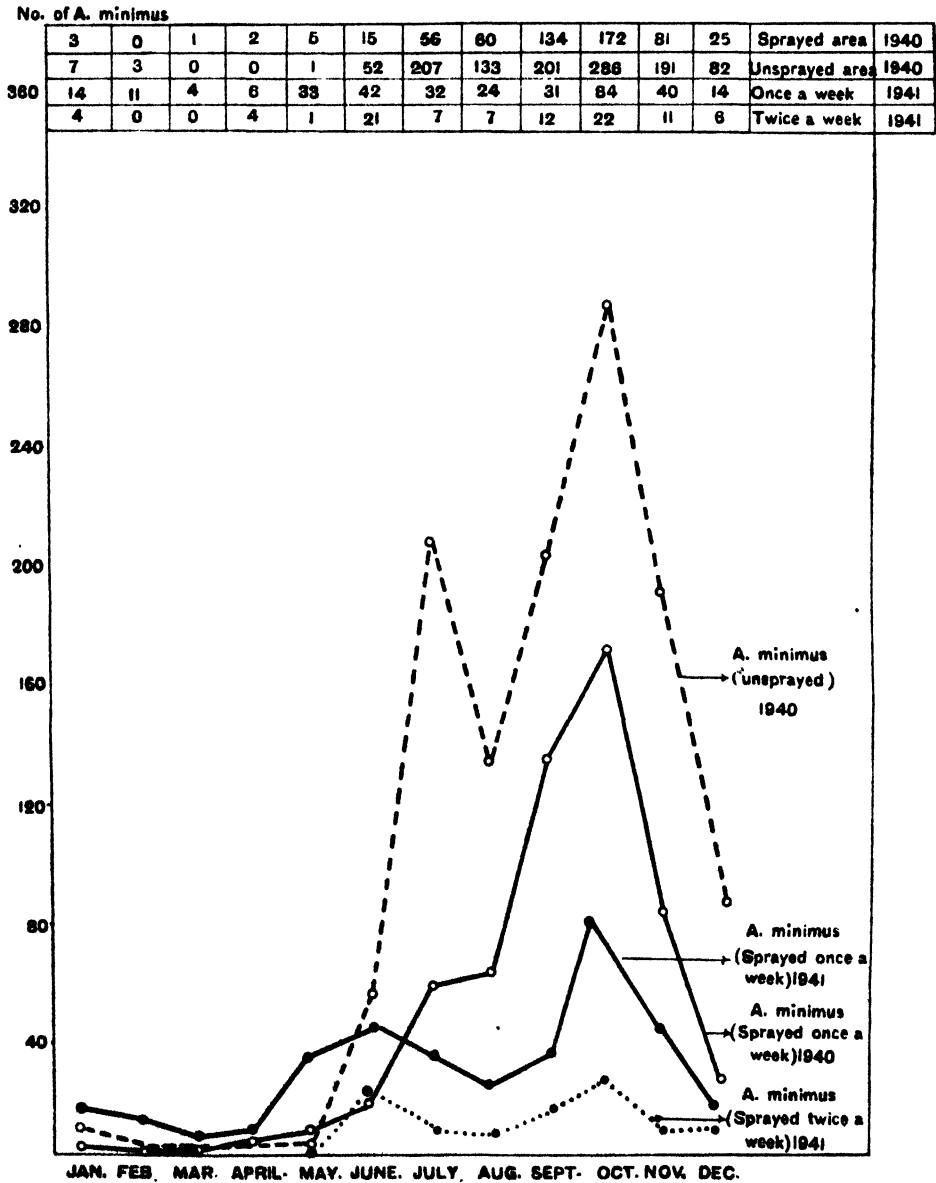


GRAPH 4.

LIMBUGURI TEA ESTATE.

Seasonal prevalence of *A. minimus* adults in 1940 and 1941.

Sprayed and unsprayed areas.





There is very definite reduction in the density of breeding in 1941 as compared with 1940, suggesting that spraying twice weekly brings about some reduction in larval output.

#### 6. DENSITY OF ADULT *A. MINIMUS* INFESTATION.

Table VI and Graph 3 show the seasonal prevalence of *A. minimus* in 1940 and 1941 respectively.

TABLE VI.

*Limbuguri Tea Estate.*

*Seasonal prevalence—A. minimus adults, 1940 and 1941.*

Year.	Jan.	Feb.	Mar.	April.	May.	June.	July.	Aug.	Sept.	Oct.	Nov.	Dec.
1940	10	3	1	2	8	67	263	193	335	458	272	107
1941	18	11	4	10	34	63	39	31	43	106	51	20

Although in 1941 there was a relatively higher adult *A. minimus* infestation in the earlier months, the numbers show a considerable reduction from June, when spraying twice weekly was introduced. This is better shown in Graph 4, which shows the density of *A. minimus* in the sprayed and unsprayed areas respectively in 1940, and the areas sprayed once weekly and twice weekly respectively in 1941.

#### 7. INFECTIVITY OF *A. MINIMUS*.

Table VII shows the infections in *A. minimus* in each of the years 1940 and 1941 in lines not sprayed, lines sprayed once weekly and lines sprayed twice weekly respectively.

TABLE VII.

*Limbuguri Tea Estate.*

*Results of spray-killing on the infectivity of A. minimus, 1940 and 1941.*

Lines.	1940.					1941.				
	Num- ber dis- sected.	NUMBER POSITIVE.			Total infect- ion index.	Num- ber dis- sected.	NUMBER POSITIVE.			Total infect- ion index.
		Gland.	Gut.	Total.			Gland.	Gut.	Total.	
Unsprayed ..	705	10	3	13	1.84					
Sprayed once weekly.	421	2	0	2	0.48	321	5	0	5	1.56
Sprayed twice weekly.						99	0	0	0	0.00
TOTAL ..	1,126	12	3	15	1.33	420	5	0	5	1.19



In 1941 the total infection index in lines sprayed once weekly was 1.56 per cent as against 0.48 per cent in 1940 in such lines, indicating a much higher degree of transmission in 1941. Despite such increased transmission, no infection was met with in 1941 in the lines sprayed twice weekly. Thus, both the results of dissections of the vector species and the malaria morbidity figures show the increased efficacy of spraying twice weekly.

#### 8. LONGEVITY OF *A. MINIMUS*.

Table VIII shows the effects of spray-killing on the longevity of *A. minimus*.

TABLE VIII.

*Effects of spray-killing on the longevity of A. minimus, 1941.*

	NUMBER AND PROPORTION OF <i>A. minimus</i> .					
	Unsprayed.		Sprayed once weekly.		Sprayed twice weekly.	
	Number.	Per cent of total.	Number	Per cent of total.	Number.	Per cent of total.
(a) <i>Wing grade.</i>						
Grade 1 ..	0	0.0	6	7.3	8	7.8
Grade 2 ..	5	9.4	36	43.9	55	52.8
Grades 1 and 2 ..	5	9.4	42	51.2	63	60.6
Grade 3 ..	33	62.3	34	41.5	32	30.8
Grade 4 ..	15	28.3	6	7.3	9	8.6
Grades 3 and 4 ..	48	90.6	40	48.8	41	39.4
(b) <i>Ovarian development.</i>						
Stage 1 ..	0	0.0	5	6.1	7	6.7
Stage 2 ..	10	18.7	26	31.7	48	46.2
Stage 3 ..	25	47.2	45	54.9	40	38.5
Stages 2 and 3 ..	35	65.9	71	86.6	88	84.7
Stage 4 ..	13	24.7	3	3.6	5	4.8
Stage 5 ..	5	9.4	3	3.6	4	3.8
Stages 4 and 5 ..	18	34.1	6	7.2	9	7.6
(c) <i>Gut content.</i>						
Fresh ..	26	49.1	47	57.3	71	68.3
Half digested ..	14	26.4	18	22.0	23	22.1
Fully digested ..	13	24.5	16	19.5	8	7.8
Empty ..	0	0.0	1	1.2	2	1.9
TOTAL ..	53	..	82	..	104	..

The results compiled in Table VIII represent the total of three examinations made during the active malaria season of 1941 in Limbuguri Tea Garden and in an adjoining tea garden where no control measures were in progress. They show that *A. minimus* with wing grades 1 and 2 represent 9.4 per cent of



the total catch in unsprayed areas, 51·2 per cent of the total catch in areas sprayed once weekly and 60·6 per cent of the total catch in areas sprayed twice weekly. Similar figures representing ovarian development are: nil stage 1, 65·9 per cent stages 2 and 3, and 34·1 per cent stages 4 and 5 in unsprayed areas; 6·1 per cent stage 1, 86·6 per cent stages 2 and 3, and 7·2 per cent stages 4 and 5 in areas sprayed once weekly; 6·7 per cent stage 1, 84·7 per cent stages 2 and 3, and 7·6 per cent stages 4 and 5 in areas sprayed twice weekly. In

TABLE IX.

*Costs of spraying and quantities used, 1940 and 1941.*

1940.		1941.	
Total costs—			
Pyrocide 20, 5·56 gallons at Rs. 43 per gallon.	Rs. 239-0-0	Pyrocide 20, 18·8 gallons	Rs. 846-0-0
Kerosene, 105·64 gallons .	Rs. 75-0-0	Kerosene, 357·2 gallons	Rs. 290-0-0
Coolies .. ..	Rs. 161-0-0	Coolies .. ..	Rs. 696-0-0
Other charges ..	Rs. 35-0-0	Other charges ..	Rs. 18-0-0
	Rs. 510-0-0		Rs. 1,850-0-0
Areas sprayed .. ..	3,742,784		11,034,361 sq. ft.
Cubic content .. ..	25,010,930		74,912,777 c.ft.
Population in sprayed area	950		2,800
Number of months sprayed	8		8
Number of sprays in each dwelling.	35	35 in about half the lines and 67 in other lines—average 46.	
Cost <i>per capita</i> for the whole season.	Re. 0-8-7		Re. 0-10-7
Cost reduced per once weekly spraying.	Re. 0-8-7		Re. 0-9-6
Cost of once spraying 1,400 sq. ft. or 100,000 c.ft.	Re. 0-3-3 $\frac{1}{2}$		Re. 0-3-11 $\frac{1}{2}$
Cost <i>per capita</i> per month	Re. 0-1-1		Re. 0-1-3 $\frac{1}{2}$
Cost reduced per once weekly spraying.	Re. 0-1-1		Re. 0-1-2 $\frac{1}{2}$
1 gallon of mixture would suffice for	225,300 c.ft.		190,100 c.ft.
or,	33,700 sq. ft.	or,	28,100 sq. ft.
1 oz. of mixture would suffice for	1,400 c.ft.		1,200 c.ft.
or,	200 sq. ft.	or,	175 sq. ft.



unsprayed areas, 24.5 per cent of the total catch exhibited fully digested gut contents, in the areas sprayed once weekly 19.5 per cent and in areas sprayed twice weekly 7.8 per cent. There is thus a marked increase in the proportion of younger anophelines in the sprayed areas, as compared with those unsprayed. The results of the experiments in 1940 and 1941 show that, while as a result of spraying once weekly *A. minimus* lives long enough to breed but not long enough to transmit, spraying twice weekly still further reduces the life span, so that besides being almost entirely unable to transmit, *A. minimus* does not even live long enough to breed in large numbers.

#### COSTS AND OTHER DATA.

Table IX shows the total costs and other data relating to spraying in 1940 and 1941 respectively. The quantities of spray are not based on any scientific basis of minimum requirements; but they represent the actual quantities consumed under the conditions of labour and supervision employed, which may be used as a working basis for control on these lines in the province of Assam.

#### SUMMARY.

- (1) The results of spray-killing during a second season are described.
- (2) Despite increased transmission in the district in 1941, the incidence of malaria was lower in 1941 than in 1940 in the experimental area.
- (3) Spleen rates were not reduced, but the average enlarged spleen showed a reduction from 9 cm. (apex to umbilicus) and 7 cm. (apex to midline) to 10 cm. (apex to umbilicus) and 8 cm. (apex to midline).
- (4) The parasite indices in children aged from 2 to 10 years and infant malaria indices showed a further decline in 1941.
- (5) In the area sprayed twice weekly no infection was met with in *A. minimus*.
- (6) Spray-killing of adult mosquitoes alters the age composition of *A. minimus*, the proportion of younger specimens being greatly increased. This is more marked in twice weekly sprayed areas than in those sprayed once a week only.
- (7) Details of cost and quantities of spray used are furnished. The cost of spraying once weekly varies from Re 0-1-1 to Re. 0-1-2½ per month *per capita*.

#### ACKNOWLEDGMENTS.

I wish to thank Lieut.-Colonel L. A. P. Anderson, I.M.S. (Retd.), Honorary Secretary of the Assam Medical Research Society, for permission to publish this paper, and Dr. F. C. McCombie, Medical Officer, and Mr. R. C. Grayburn, Manager, Limbuguri Tea Estate, for affording facilities to carry out the experiment.

#### REFERENCE.

- VISWANATHAN, D. K. (1941) .. Experimental malaria control in a hyperendemic tea garden in Upper Assam by the use of pyrethrin 20 as an insecticidal spray. *J. Mal. Inst. Ind.*, **4**, 1, pp. 35-55.







OBSERVATIONS ON THE SWARMING AND PAIRING OF  
*A. SUNDAICUS* (RODENWALDT) AND *A. SUBPICTUS*  
(GRASSI).

BY

V. VENKAT RAO, M.R.SAN.I. (London)  
(*Chief Malaria Inspector*),

B. B. ROY  
(*Malaria Inspector*),

AND

P. JAGANNADHA RAO  
(*Malaria Sub-Inspector*).

(*Bengal-Nagpur Railway, Calcutta.*)

[February 5, 1942.]

It is stated by Rao and Russell (1938) that, prior to the publication of their observations on the swarming and pairing of mosquitoes, there was no literature dealing with this subject in India. We, therefore, consider it worth while to record our field observations on the swarming and pairing of *A. sundaicus* and *A. subpictus*.

These observations were made at the railway station of Gangadharpur in Orissa, situated 36 miles south of Khurda Road junction on the east coast section of the Bengal-Nagpur Railway. This station is about two miles from Nairi village on the Chilka Lake, around which malaria is practically endemic (Senior White and Adhikari, 1939). The main vector in this area is *A. sundaicus*, though *A. annularis* and *A. aconitus* have been found infected and infective on a few occasions (Senior White and Adhikari, *loc. cit.*). Owing to a severe outbreak of malaria in stations along the Chilka coast, it was found necessary to bring this station under control, along with four others, in October 1941. The method of control adopted is the destruction of adult mosquitoes by house spraying with pyrethroid 20.



Routine weekly adult catches made in the station and staff quarters subsequent to the institution of control measures revealed the presence of *A. sundaicus* in very large numbers, the hourly catch at times rising to as much as 100 adults of this species. Its density during this season has been far greater than that observed in any previous year.

On the evening of January 10, 1942, the authors were walking on the station platform when a large swarm of mosquitoes was observed. It was then exactly 5.40 p.m. (Indian Standard Time). The sun was just setting, but a grey cloud on the southern horizon was lit up by the setting sun, and against this as a background, the mosquitoes could be clearly seen. Closer observation showed that pairing was also taking place.

It is not possible to say how long swarming had been in progress, but it could not have commenced much earlier than 5.30 p.m., as our subsequent observations showed. The number of mosquitoes composing the swarm was estimated to be about 5,000. The swarm appeared directly in front of us above our heads, about six feet from the ground level; its vertical measurement was about one foot, its length about ten feet, and its width about four feet. All the mosquitoes were making dancing movements, which were both circular and up and down. Perhaps their movements were necessarily restricted to a few inches owing to the size of the swarm. But in the rapidly disappearing twilight, it was not possible to make accurate observations of the movements of individual mosquitoes in the swarm.

As *A. sundaicus* constituted the majority of our routine adult catches, we made collections while the swarming was in progress in order to arrive at a rough estimate of the density of this species. The ordinary adult catching tube was used for this purpose, and was moved to and fro in the swarm for exactly a minute, during which time the mosquitoes were being sucked into it. The catch, which was identified the next morning, was composed as follows:—

<i>A. subpictus</i>	..	3 ♂	3 ♀
<i>A. sundaicus</i>	..	33 ♂	27 ♀

The swarm began to thin out at 6 p.m., and was completely invisible to us about ten minutes afterwards owing to darkness.

Unfortunately, it was not possible to carry out observations as to the degree of light intensity and wind velocity at the time of swarming.

The phenomenon was again observed on January 20, at 5.30 p.m. On this occasion, relatively few mosquitoes were seen to swarm, but more arrived in groups up to about 5.50 p.m. Instead of a large swarm at one place, several groups, apparently isolated and consisting each of a few hundreds, were observed all along the southern half of the platform. A catch made in one group for exactly one minute yielded the following mosquitoes:—

<i>A. subpictus</i>	..	2 ♂	1 ♀
<i>A. sundaicus</i>	..	8 ♂	12 ♀

We do not consider this reduced catch indicates a reduction in the density of mosquitoes, since it is possible to catch a much larger number of mosquitoes from a thick group than from a thin group. Swarming was, on this occasion, observed to continue till 6.10 p.m., when darkness prevented us from making further observations.



On both occasions, pairing was observed. During the dance, two mosquitoes would suddenly meet and place themselves in the required position with the tips of abdomens in contact and usually with legs interlocked. Then, they would emerge from the group and, after a transient period, separate themselves. A number of copulating pairs were observed by all of us and, in almost every case, the pairs came out of the group during copulation and separated themselves outside. Some of them, after separation, were observed to resume their part in the dance.

After several vain attempts, we succeeded in catching two pairs during copulation. One of these proved to be *A. subpictus* and the other *A. sundaicus*.

The mosquitoes caught during the dance were found to be mostly fresh unrubbed specimens, and all the female specimens examined were found to have fed.

The main breeding places of *A. sundaicus* in this area are the Chilka Lake and tanks, *jheels* and borrowpits on the foreshore (Senior White and Adhikari, *loc. cit.*). The margin of the lake is about two miles away from this station. There are in the vicinity three tanks and several borrowpits which contain more or less fresh water. The unusual density of *A. sundaicus* in the station led us to believe that breeding must be going on in the tanks and borrowpits. We, therefore, made a careful search for larvæ in local water collections as well as in the lake. The results are shown in the table:—

TABLE.

Breeding place.	Time occupied in larval collection (hours).	<i>A. subpictus</i> .	<i>A. vagus</i> .	<i>A. sundaicus</i> .	<i>A. hyrcanus</i> .	<i>A. jeyporiensis</i> .
Tanks and borrowpits.	1½	4*	2	..	12	1
Chilka Lake † ..	3	37 ♂ 30 ♀	0 ♂ 4 ♀	232 ♂ 287 ♀	..	..

\* Confirmed by results of hatching.

† Since larvæ were collected in very large numbers, preliminary examination for elimination of the non-*subpictus*-cum-*sundaicus* group was not possible. Therefore, all adults hatching out within two days of the larval collection were caught and identified. As this furnished the required information regarding the species breeding in the lake and their respective density, the remaining larvæ and pupæ were discarded.

Our observations show that the flight range of this species, both male and female, is at least two miles, a factor of considerable importance in the formulation of malaria control measures.

The question which now arises is, why the mosquitoes should disperse as far as two miles from their breeding place for swarming and mating? It is well known that *A. sundaicus* has a much longer flight range than many other *Anopheles*, but the spleen rates recorded in a number of villages situated at varying distances from the shore of the lake by Senior White and Adhikari (*loc. cit.*) show that, while *A. sundaicus* can fly for over two miles, it does not ordinarily fly so far. Is the density of this mosquito at present so unusually



heavy that swarming extends over the wide area between the lake and the railway station? In spite of the improbability of this suggestion, one of us walked nearly three furlongs from the station towards the lake on January 20 to verify it, but no swarming was observed. Is it possible that the tendency of mosquitoes to swarm in large numbers in the station is due to the radiation of heat from the raised stone platform and the nearby range of hills?

### SUMMARY.

1. A description of the swarming and pairing of *A. subpictus* and *A. sundaicus* is given.

2. A group of mosquitoes including more than one species may swarm together, but pairing takes place only between individuals of the same species.

3. It has been observed that, in most cases, the copulating pairs emerge from the group and separate outside it, though they may subsequently resume the dance.

4. Observations on the larval collections made in the breeding places in the area are recorded.

5. The flight range of *A. sundaicus* is indicated by these observations and the reasons why the swarming should take place far away from the breeding place are discussed.

### ACKNOWLEDGMENT.

The authors wish to thank Captain R. Senior White, Malariologist, Bengal-Nagpur Railway, for suggesting the lines on which this paper should be written and for permitting its publication.

### REFERENCES.

- RAO, T. R., and RUSSELL, P. F. *J. Mal. Inst. Ind.*, **1**, 4, p. 395.  
(1938).  
SENIOR WHITE, R., and ADHIKARI, *Ibid.*, **2**, 4, p. 395.  
A. K. (1939).



## THE CONTROL OF MOSQUITO-BREEDING IN CANAL DISTRIBUTARIES BY GROWING CERTAIN PLANTS ON THEIR BANKS.

BY

R. BHASKER RAO, M.B., B.S., B.S.SC.  
(Health Officer),

AND

H. RAMOO, B.A.  
(Entomological Assistant).  
(Antimalarial Scheme, Pattukkottai, Madras Presidency.)

[February 12, 1942.]

THE observations recorded in this paper were carried out in Pattukkottai Taluk, Tanjore District, Madras Presidency, where *A. culicifacies* is the sole malaria carrier of any practical importance.

Whilst making routine larval collections in the branch irrigation canals in this area, it was often noticed that the intensity of breeding of this species was relatively greater in canals whose banks were devoid of shrubs, creepers, etc., than in those where there was a dense marginal growth of such plants. The plant which grows most abundantly on the canal banks in this area is *Ipomea biloba*, a fast growing creeper with broad bilobed foliage, the stems and leaves of which project a few inches, and sometimes a few feet, over the water surface. These were planted by the Public Works Department along the embanked portions of the canal, apparently for the purpose of strengthening the bunds. They are commonly used in sandy places as sand binders. In 1939 two sections of the canal were selected for observation, one with a dense growth of the creeper and the other with clear edges. The distance between these two sections was about 100 to 150 yards and each was practically equidistant from the nearest dwelling houses. Routine larval collections were made from July 1939 to January 1940. The intensity of breeding of *A. culicifacies* in the section devoid of creepers was nearly three times as great as in that where they were present (Table I). It was also noticed that in the latter area the majority of the larvæ caught were in the 3rd or 4th instar. This suggests that there was







probably very little oviposition taking place in this section, a considerable proportion of the larvæ present having been carried down by drift from the higher reaches of the canal.

It was, therefore, decided to attempt the control of marginal breeding of *A. culicifacies* in the canals by growing suitable plants. The following were tried :—

1. *Tithonia diversifolia*.
2. *Artemisia vulgaris* (Mugwort).
3. *Oscimum sanctum* and a few other members of this group.
4. *Adothoda basia*.
5. *Vitex negundo*.

The first two did not come up at all, possibly because they require a certain altitude and a well-drained soil. The third group consists of herbs with a strong aromatic odour, and it was thought possible that this might exert an additional effect as a repellent; but though they were present in abundance in waste lands in this neighbourhood, they did not grow well near the water edge. *Adothoda basia*, a fast growing shrub with broad leaves, also yielded disappointing results. When planted close to the water edge, all its leaves were found to be shrunken and the growth of the plant was retarded. *Vitex negundo* is a quick growing perennial downy tree or shrub, which throws out lateral branches with thick quinque-foliolate leaves. This was found to grow very well near the water edge, and its dense foliage was capable of providing both shade and mechanical obstruction to the oviposition of mosquitoes. The leaves possess a strong aromatic odour which may exert some repellent effect on egg-laying mosquitoes. This plant is called *Nochchi* in Tamil and belongs to the family Verbenaceæ, the members of which are principally confined to the tropics. The irrigation authorities of the Public Works Department are not averse to the planting of these shrubs on the canal margins, as they believe that they serve to strengthen the bunds. They possess the further advantage over creepers that they do not hide erosions from view. *Vitex negundo* is easily propagated by means of small cuttings which grow into separate plants. During the first year, it was planted along a short length of the main irrigation canal which passes through this town. Observations were made in 1940 regarding the intensity of marginal breeding of *A. culicifacies* in the sections where it was growing as compared with canals with clear margins. The two sections selected were about 200 yards apart and were equidistant from the nearest habitations. Table II gives a record of routine larval collections made during 1940. As it took some time for the plants to attain a reasonable growth, no observations were made until the middle of August. No collections were made after October, as the canals were put on turn from that month. It was thought that no useful purpose would be served by continuing our observations during this period, owing to the considerable and frequent fluctuations in water level. Further observations were made in 1941 also, by which time the plants had grown up more densely. Two sections where *Vitex negundo* was growing and two contrast sections were selected. The results obtained confirmed those previously recorded, the number of larvæ collected from the sections planted with *Vitex negundo* being very scanty compared with those from the contrast sections (Tables III and IV).



TABLE II.

Date of collection.	<i>Viter negundo</i> SECTION.				CLEAR (CONTRAST) SECTION.			
	Num-ber of anophe- line larvæ per collec- tion.	Num-ber of larvæ identi- fied.	Species identified.		Num-ber of anophe- line larvæ per collec- tion.	Num-ber of larvæ identi- fied.	Species identified.	
			<i>A. culici- facies.</i>	<i>A. sub- pictus.</i>			<i>A. culici- facies.</i>	<i>A. sub- pictus.</i>
14.viii.40	4	3	3	..	21	16	16	..
20.viii.40	..	..	..	..	7	5	5	..
30.viii.40	5	5	1	4	6	4	2	2
6.ix.40	..	..	..	..	17	12	12	..
14.ix.40	2	2	1	1	13	12	11	1
22.ix.40	..	..	..	..	12	8	7	1
27.ix.40	..	..	..	..	19	14	11	3
1.x.40	..	..	..	..	61	47	41	6
5.x.40	2	2	1	1	4	3	3	..

Note.—The time taken for each collection was constant.



TABLE III.

Date of collection.	Vitex negundo SECTION A.				CLEAR (CONTRAST) SECTION A.			
	Num-ber of anophe-line larvæ per collec-tion.	Num-ber of larvæ identi-fied.	Species identified.		Num-ber of anophe-line larvæ per collec-tion.	Num-ber of larvæ identi-fied.	Species identified.	
			<i>A. culici-facies.</i>	<i>A. sub-pictus.</i>			<i>A. culici-facies.</i>	<i>A. sub-pictus.</i>
1.vii.41	1	1	1	..	17	14	12	2
9.vii.41	1	1	1	..	8	6	6	..
18.vii.41	..	..	..	..	13	10	10	..
26.vii.41	1	1	1	..	23	18	14	4
2.viii.41	..	..	..	..	20	16	16	..
8.viii.41	1	1	..	1	15	11	11	..
15.viii.41	2	2	..	2	14	9	9	..
23.viii.41	2	2	..	2	35	27	21	6
28.viii.41	3	2	..	2	24	18	18	..
4.ix.41	1	1	..	1	34	23	14	9
11.ix.41	..	..	..	..	19	16	13	3
18.ix.41	..	..	..	..	22	15	12	3
26.ix.41	..	..	..	..	15	11	11	..
4.x.41	..	..	..	..	4	2	2	..
11.x.41	..	..	..	..	1	1	1	..
27.x.41	..	..	..	..	..	..	..	..

Note.—The time taken for each collection was constant.



TABLE IV.

Date of collection.	Viter negundo SECTION B.					CLEAR (CONTRAST) SECTION B.			
	Num-ber of anophe- larvæ per collec- tion.	Num-ber of larvæ identi- fied.	Species identified.			Num-ber of anophe- larvæ per collec- tion.	Num-ber of larvæ identi- fied.	Species identified.	
			<i>A. culici- facies.</i>	<i>A. sub- pictus.</i>	<i>A. varuna.</i>			<i>A. culici- facies.</i>	<i>A. sub- pictus.</i>
1.viii.41	4	4	4	..	..	11	8	8	..
9.vii.41	2	2	2	..	..	9	5	5	..
18.vii.41	..	..	..	..	..	4	3	3	..
26.vii.41	3	2	2	..	..	16	11	11	..
2.viii.41	1	1	1	..	..	31	26	26	..
8.viii.41	..	..	..	..	..	15	11	11	..
15.viii.41	1	1	..	..	1	18	13	12	1
23.viii.41	1	1	..	..	1	21	16	13	3
28.viii.41	2	2	2	..	..	44	32	25	7
4.ix.41	1	1	1	..	..	25	19	17	2
11.ix.41	..	..	..	..	..	..	..	..	..
18.ix.41	..	..	..	..	..	17	13	13	..
26.ix.41	..	..	..	..	..	9	7	7	..
4.x.41	1	1	..	..	1	1	1	1	..
11.x.41	..	..	..	..	..	..	..	..	..
27.x.41	..	..	..	..	..	13	8	8	..

Note.—The time taken for each collection was constant.



SUMMARY.

The effect of planting higher vegetation such as *Vitex negundo* along the margins of canals on the breeding of *A. culicifacies* is recorded. This method is recommended for controlling this species where other measures are impracticable.

ACKNOWLEDGMENT.

We wish to thank Dr. R. Adiseshan, Director of Public Health, Madras, for permission to publish this note.







# ON THE IMPORTANCE OF *ANOPHELES PALLIDUS* AS A CARRIER OF MALARIA IN UDAIPUR STATE, CENTRAL PROVINCES.

BY

D. N. ROY

AND

T. C. BISWAS

(Department of Medical Entomology, School of Tropical Medicine, Calcutta.)

[March 12, 1942.]

UDAIPUR STATE, a hilly tract in the Eastern States Agency, is situated approximately 150 miles from Chaibassa, the administrative centre of Singbhum District. During a malaria survey of Dharamjaigarh, the capital of the State, which was carried out during the period August 18 to November 2, 1941, the spleen index in children was found to be 60·8 per cent, denoting a condition of hyperendemicity.

The mosquitoes which were collected either as adults or larvæ belonged to the following species: *Anopheles vagus*, *A. fluviatilis*, *A. pallidus*, *A. culicifacies*, *A. subpictus*, *A. jeyporiensis*, *A. ramsayi*, *A. hyrcanus*, *A. aconitus* and *A. barbirostris*. Of these, *A. pallidus* and *A. culicifacies* comprised the largest proportion of adult catches. The results of adult collections by months are given in Table I.

TABLE I.

Monthly prevalence of adult anophelines (female) of different species.

Month, 1941.	<i>A. vagus</i> .	<i>A. fluviatilis</i> .	<i>A. pallidus</i> .	<i>A. culicifacies</i> .	<i>A. subpictus</i> .	<i>A. jeyporiensis</i> .	<i>A. aconitus</i> .	<i>A. annularis</i> .
August 18-31	40	18	155	80	103	0	0	0
September	1	5	347	361	10	14	1	0
October ..	0	7	340	503	2	18	0	0
November 1-2.	0	8	12	25	0	0	0	3



Malaria transmission has been extensively studied in the Eastern Satpura Ranges and the Singhbhum Hills, the former of which is much closer to Udaipur State than the latter. In the Eastern Satpuras, *A. culicifacies* was reported to be the vector (Senior White and Adhikari, 1940), and in the Singhbhum Hills, *A. fluviatilis*, *A. varuna* and *A. minimus* (Senior White and Das, 1938).

It seemed, therefore, probable that either *A. culicifacies* or one of the *fluviatilis* group would prove to be the vector in Dharamjaigarh. The results of anopheline dissections, however, did not wholly substantiate this supposition (Table II).

TABLE II.  
*Results of mosquito dissection.*

Species.	Number dissected.	Number caught in dwelling houses.	Number showing sporozoites in salivary glands.	Number caught in cattle-sheds.	Number showing sporozoites in salivary glands.	Sporozoite rate.
<i>A. pallidus</i> ..	854	719	6	135	0	0.7
<i>A. culicifacies</i> ..	969	881	5	88	0	0.5
<i>A. fluviatilis</i> ..	38	38	0	0	0	0.0
<i>A. subpictus</i> ..	115	105	0	10	0	0.0
<i>A. vagus</i> ..	41	34	0	7	0	0.0
<i>A. jeyporiensis</i> ..	32	31	0	1	0	0.0
<i>A. aconitus</i> ..	1	1	0	0	0	0.0
<i>A. annularis</i> ..	3	0	0	3	0	0.0

The infection rates of 0.7 and 0.5 in *A. pallidus* and *A. culicifacies*, respectively, indicate that both should be regarded as vectors of major importance.

The dates on which the infected specimens were collected were:—

*A. pallidus*:

August	18	2
"	21	1
"	28	1
September	1	1
"	29	1

*A. culicifacies*:

September	12	1
"	20	1
October	2	1
"	11	1
"	29	1



These findings suggest that *A. pallidus* is responsible for the transmission of malaria during the early part of the monsoon, and *A. culicifacies* during the latter part of the rainy season and autumn.

Previous records of *A. pallidus* acting as a carrier of malaria are given in Table III.

TABLE III.  
*Records of dissection of A. pallidus.*

Author.	Locality.	Number dissected	Oöcyst rate.	Sporozoite rate.	REMARKS.
Sur and Sur (1929)	Krishnagar (Bengal).	1,232	..	0.24	
Wats (1924) ..	Singhbhum Hills.	..	..	..	Out of 556 anophelines, sporozoites were found in only one specimen of <i>A. fuliginosus</i> .
Timbres (1935) ..	Birbhum (Bengal).	27,238	..	0.03	
Iyengar (1939) ..	Birbhum (Bengal).	254	0.8	..	
Senior White ..	Orissa, Singhbhum and Hazaribagh.	7,513	0.8	..	Private communication.

A small number of precipitin tests with human and bovine antisera were carried out in order to obtain some indication as to the preferential feeding habits of *A. pallidus* and *A. culicifacies*. Among the mosquitoes in which sporozoites were found in the salivary glands, four *A. pallidus* and four *A. culicifacies* showed the presence of blood in their stomach (visible externally). Out of five specimens of *A. pallidus* subjected to precipitin tests, four reacted to human and none to bovine antiserum. Four specimens of *A. culicifacies*, on the other hand, gave positive reactions with bovine and were negative to human antiserum.

Although no definite information is available as to the interval between the ingestion of a blood meal and egg-laying in *A. pallidus* in particular, it may be supposed that in this respect this species behaves in the same way as *A. annularis* and *A. subpictus*, in both of which the development of eggs covers a period of 96 hours (Roy, 1940). It, therefore, follows that those specimens of *A. pallidus* in which sporozoites were detected had taken at least three blood meals prior to their capture. The first and the third meals were definitely of human origin, and it would be interesting to conduct further studies on the feeding habits of zoophilic mosquitoes with a view to determining whether such a species when once diverted to man acquires a preference for feeding exclusively on human beings.



# SUMMARY.

Two species of anophelines, *A. pallidus* and *A. culicifacies*, were found to be vectors in Dharamjaigarh in Udaipur State.

# ACKNOWLEDGMENT.

We wish to acknowledge the assistance received from Mr. R. Senior White, Malariologist, B.-N. Railway, in the compilation of this note.

# REFERENCES.

- |   |     |  |
|---|-----|--|
| IYENGAR, M. O. T. (1939)                      | ..  | <i>J. Mal. Inst. Ind.</i> , <b>2</b> , p. 105.   |
| ROY, D. N. (1940)                             | ..  | <i>Ibid.</i> , <b>3</b> , p. 499.  |
| SENIOR WHITE, R., and ADHIKARI, A. K. (1940). |     | <i>Ibid.</i> , <b>3</b> , p. 383.  |
| SENIOR WHITE, R., and DAS, B. K. (1938).      |     | <i>Ibid.</i> , <b>1</b> , p. 169.  |
| SUR, S. N., and SUR, P. (1929)                | ... | <i>Report of the Bengal Field Malaria Research Krishnagar Laboratory, 1926-28.</i> Calcutta : Bengal Govt Press. |
| TIMBRES, H. G. (1935)                         | ..  | <i>Rec. Mal. Surv. Ind.</i> , <b>5</b> , p. 345.   |
| WATS, R. C. (1924)                            | ..  | <i>Malaria survey of the mining settlements of Singhbhum (Orissa). Report No. II.</i> Patna : Govt. Press.       |



# ABSTRACT.

## MALARIA IN THE TISTA VALLEY, DARJEELING DISTRICT\*.

BY

BHUPENDRA MOHAN KHAN

(Assistant Epidemiologist, Bengal Public Health Department).

[February 21, 1942.]

A FIELD laboratory under the Bengal Public Health Department was established at Kalimpong in May 1940 with the object of establishing the identity of the vector species of anopheline in the Tista and neighbouring valleys in Darjeeling District. Previous investigations in this area had shown that malaria was prevalent up to a height of 3,500 feet above sea-level.

During the year 1941, 3,487 anopheline mosquitoes of the following species were dissected :—

Species.	Number dissected.
<i>A. aconitus</i> .. .. .	44
<i>A. annularis</i> .. . . .	779
<i>A. culicifacies</i> .. . . .	79
<i>A. fluviatilis</i> .. . . .	11
<i>A. karwari</i> .. . . .	2
<i>A. lindesayi</i> .. . . .	9
<i>A. maculatus</i> .. . . .	1,725
<i>A. maculipalpis</i> .. . . .	48
<i>A. minimus</i> .. . . .	94
<i>A. subpictus</i> .. . . .	24
<i>A. vagus</i> .. . . .	475
<i>A. willmori</i> .. . . .	197

Negative results were recorded from all species except *A. minimus*, of which 5 were found infected (salivary glands 3, gut 2). All the infected mosquitoes were collected at Karmatar, which is situated 15 miles from Darjeeling at an elevation of 1,200 feet.

G. C.

\* A copy of the original manuscript has been placed in the Library of the Malaria Institute of India, Kasauli. This is available on loan to workers who wish to consult it. (Editor.)







## ABSTRACT.

### MALARIA IN PURI\*.

BY

R. G. PANIGRAHI, M.B., B.S., D.P.H.  
(Provincial Malaria Officer, Orissa).

[March 20, 1942.]

A MALARIA SURVEY of Puri, a town with a population of 37,500 situated on the shore of the Bay of Bengal 58 miles north of Cuttack and 311 miles south of Calcutta, was carried out during the period April 1, 1940 to March 31, 1941.

Malaria incidence in Puri is normally slight, but the town is subject to periodical epidemics at irregular intervals. Outbreaks of varying intensity occurred in 1925, 1933, 1936 and 1940, which were years of abnormally heavy rainfall. The seasonal incidence of these epidemics is from August to December, the peak usually occurring in November. In some years there is also evidence of a minor rise in malarial incidence in March or April.

Spleen examinations showed that the distribution of malaria in the municipality is extremely irregular, the rates ranging from 3 per cent in one ward to approximately 70 per cent in another being recorded (Table I).

Fifteen species of anophelines were collected either in the adult or larval stage (Tables II and III). The most numerous species were *A. annularis*, *A. hyrcanus*, *A. vagus*, *A. ramsayi* and *A. subpictus*. *A. sundaicus* constituted 1.8 per cent of the total adult and 0.3 per cent of the total larval collections. The highest salinity in which this species was found breeding was 42 parts per 100,000.

As the result of 17,423 mosquito dissections, infections were recorded in *A. sundaicus* (oöcyst rate 0.8 per cent, sporozoite rate 1.1 per cent) and *A. annularis* (oöcyst rate 0.05 per cent, sporozoite rate 0.08 per cent). A single gut infection of *A. ramsayi* was detected out of a total of 1,658 dissected. All

---

\* A copy of the original manuscript has been placed in the Library of the Malaria Institute of India, Kasauli. This is available on loan to workers who wish to consult it. (Editor.)



the infections detected were in mosquitoes collected during the months of September to December inclusive. The results of dissections of *A. sundaicus* and *A. annularis* by months and wards are given in Tables IV and V.

The author considers that *A. sundaicus* is the principal vector of malaria, whilst *A. annularis* plays a secondary rôle. He thinks it probable that the latter species only becomes infected in years when an epidemic has been started by *A. sundaicus*.

It is considered that malaria in Puri could be controlled without great difficulty by keeping the tanks, ponds, pools and borrowpits free from aquatic vegetation and treating the margins of these breeding places with larvicides. A nala which ends blindly near the shore and receives a considerable amount of sullage needs special engineering treatment.

G. C.

TABLE I.  
*Spleen examinations.*

Name of ward.	APRIL-MAY 1940.			MARCH 1941.		
	Number examined.	Number with enlarged spleen.	Spleen rate.	Number examined.	Number with enlarged spleen.	Spleen rate.
Noliasahi .. ..	120	3	2.5	120	3	2.5
Mangalaghat ..	60	16	26.6	50	21	42.0
Kumarpara .. ..	150	34	22.6	150	60	40.0
Matipara .. ..	50	35	70.0	50	30	60.0
Tikerpara .. ..	70	43	61.4	60	36	60.0
Narsinghallavpatna ..	115	33	28.7	100	42	42.0



TABLE II.  
Total anopheline adult collection, Puri Municipality.

Month.	<i>A. sundacus.</i>	<i>A. subpictus.</i>	<i>A. vagus.</i>	<i>A. culicifacies.</i>	<i>A. annularis.</i>	<i>A. pallidus.</i>	<i>A. ramsayi.</i>	<i>A. jamesi.</i>	<i>A. hyrcanus.</i>	<i>A. barbrostris.</i>	<i>A. varuna.</i>	<i>A. aconitius.</i>	<i>A. philippinensis.</i>	<i>A. tessellatus.</i>	<i>A. splendius.</i>	Total.
1940.																
April ..	..	89	26	1	34	10	3	..	36	1	..	1	..	..	..	201
May ..	..	320	60	2	96	11	15	..	51	4	5	8	1	..	..	573
June ..	1	208	77	8	642	15	111	..	189	4	..	1	3	..	..	1,259
July ..	..	958	167	67	193	3	226	..	362	29	..	1	1	..	..	2,007
August ..	52	715	538	155	1,711	5	720	1	527	22	3	17	2	..	..	4,468
September ..	163	541	452	142	2,696	12	319	..	581	25	7	8	1	..	..	4,947
October ..	292	408	1,970	77	2,769	13	667	..	847	12	4	21	..	3	..	7,063
November ..	135	216	1,095	30	3,365	37	981	..	1,781	28	56	131	..	2	..	7,857
December ..	71	107	350	11	3,208	41	672	..	1,138	10	86	100	3	9	..	5,806
1941.																
January ..	16	66	573	15	2,603	20	600	1	780	3	64	75	4	17	1	4,838
February ..	75	110	677	8	1,491	20	225	..	510	..	79	47	1	8	1	3,252
March ..	26	483	725	1	1,169	10	77	..	324	2	12	10	2	2	..	2,843
Total ..	831	4,221	6,710	517	19,977	197	4,616	2	7,126	140	316	420	18	41	2	45,134
PERCENTAGE	1.8	9.3	14.9	1.1	44.3	0.4	10.2	0.0	15.8	0.3	0.7	0.9	0.04	0.09	0.0	..



TABLE III.  
Monthly collection of anopheline larvæ, Puri Municipality.

Species.	1940.										1941.				
	May.	June.	July.	Aug.	Sept.	Oct.	Nov.	Dec.	Total.	Percentage.	Jan.	Feb.	Mar.	Total.	Percentage.
<i>A. sundaiacus</i>	..	..	..	..	31	16	38	1	86	0.4	15	7	1	23	0.1
<i>A. subpictus</i>	436	505	394	465	434	338	420	177	3,169	14.5	208	605	1,117	1,930	12.3
<i>A. vagus</i>	85	127	104	357	293	373	668	375	2,382	11.0	606	1,173	2,345	4,124	26.3
<i>A. culicifacies</i>	2	15	12	41	132	148	91	7	448	2.1	5	2	14	21	0.1
<i>A. varuna</i>	2	1	2	9	13	4	4	..	35	0.2	27	23	40	90	0.6
<i>A. aconius</i>	..	..	1	6	..	1	2	1	11	0.1	23	11	31	65	0.4
<i>A. annularis</i>	12	174	38	266	536	702	770	271	2,769	12.8	526	504	493	1,523	9.7
<i>A. pallidus</i>	13	9	17	10	14	4	18	3	88	0.4	31	11	27	69	0.4
<i>A. philippinensis</i>	5	16	16	10	38	10	..	1	96	0.4	5	2	5	12	0.08
<i>A. ramsayi</i>	32	273	205	887	540	727	681	599	3,944	18.2	557	542	269	1,368	8.7
<i>A. hyrcanus</i>	103	725	920	1,662	1,933	907	1,318	878	8,446	39.0	1,700	2,525	2,151	6,376	40.7
<i>A. barbirostris</i>	..	1	53	30	50	18	21	29	202	0.9	16	38	9	63	0.4
<i>A. splendidus</i>	..	..	..	..	..	..	..	..	..	..	1	..	..	1	0.0
<b>Total</b>	..	..	..	..	..	..	..	..	21,676	..	..	..	..	15,665	..



TABLE IV.  
Dissections of *A. sundaicus*, *Puri Municipality*.

Month.	MANGALAGHAT.		KUMARPARA.		TIKERPARA.		MATIAPARA.		NARSINGHABALIAP- PATNA AND CHAKRATIRTHA, KUMUTIPATNA. ETC.				Total.
	Number dissected.	Number with gut +	Number dissected.	Number with gut +	Number dissected.	Number with gut +	Number dissected.	Number with gut +	Number dissected.	Number with gut +	Number with gut +	Number with gland +	
Total catches.													
1940.													
April ..	..	..	..	..	..	..	..	..	..	..	..	..	..
May ..	..	..	..	..	..	..	..	..	..	..	..	..	..
June ..	..	..	..	..	..	..	..	..	..	..	..	..	..
July ..	..	..	..	..	..	..	..	..	..	..	..	..	..
August ..	..	..	..	..	..	..	..	..	..	..	..	..	..
September ..	6	50	..	..	1	..	..	..	42	1	..	..	42
October ..	110	263	7	..	..	..	..	..	80	2	..	..	107
November ..	18	120	14	1	..	..	..	..	119	0	..	..	240
December ..	3	64	3	0	..	..	..	..	54	0	..	..	89
1941.									48	0	..	..	55
January ..	5	15	1	0	..	..	..	..	8	0	..	..	14
February ..	40	68	..	..	..	..	..	..	6	0	..	..	46
March ..	15	23	..	..	..	..	..	..	3	0	..	..	24
Total ..	197	748	1	1	25	1	2	7	360	3	4	23	617
INFECTION RATE.	..	0.5	..	4.0	..	8.0	..	..	..	0.8	1.1	..	Oöcyst rate 0.8 Sporozoite rate 1.1



TABLE V.

*Dissections of A. annularis, Puri Municipality.*

Month.	Total catches.	MANGALAGHAT.			KUMARPARA.			TIKERPARA.			MATIAPARA.			NARSINGHBALLAV-PATNA AND KUMUTIPATNA.			RAILWAY COLONY AND CHAKRATIRTHA, ETC.			Total.
		Number dissected.	Number with gut +	Number with gland +	Number dissected.	Number with gut +	Number with gland +	Number dissected.	Number with gut +	Number with gland +	Number dissected.	Number with gut +	Number with gland +	Number dissected.	Number with gut +	Number with gland +	Number dissected.	Number with gut +	Number with gland +	
1940.																				
April	33	17	0	0	11	0	0	20	0	0	1	0	0	..	0	0	1	0	0	33
May	94	32	0	0	30	0	0	39	0	0	..	0	0	..	0	0	8	0	0	94
June	585	35	0	0	57	0	0	205	0	0	60	0	0	145	0	0	2	0	0	501
July	186	148	0	0	12	0	0	40	0	0	40	0	0	24	0	0	6	0	0	157
August	1,579	179	0	0	125	0	0	132	0	0	276	0	0	502	0	0	23	0	0	1,206
September	2,452	148	0	0	129	0	0	99	0	0	183	0	0	508	0	0	5	0	0	1,103
October	2,577	219	0	0	159	0	0	80	0	0	76	1	0	375	0	0	2	0	0	840
November	3,249	109	0	1	144	1	1	199	0	1	152	0	0	386	2	1	..	..	..	1,100
December	3,137	109	0	0	284	0	1	182	0	1	170	1	1	346	0	0	..	..	..	1,091
1941.																				
January	2,533	117	0	0	528	0	0	438	0	0	199	0	0	221	0	0	..	..	..	1,503
February	1,460	73	0	0	357	0	0	134	0	0	66	0	0	69	0	0	..	..	..	699
March	1,140	17	0	0	346	0	0	295	0	0	66	0	0	45	0	0	..	..	..	769
Total	19,025	1,094	0	1	2,182	1	2	1,863	0	2	1,289	2	1	2,621	2	1	47	0	0	9,096
INFECTION RATE.	..	..	0.09	0.05	..	0.05	0.09	..	..	0.01	..	0.15	0.08	0.08	0.04	..	..	..	..	Oöcyst rate 0.05 Sporozoite rate 0.08



## ON A NEW VARIETY OF *ANOPHELES TURKHUDI* FROM PALESTINE.

BY

Z. SALITERNIK

(*District Inspector, Bureau of Antimalaria Control*),

AND

O. THEODOR

(*Medical Entomologist, Department of Parasitology, Hebrew University*).

[April 14, 1942.]

On 2.i.1940 two mosquitoes were caught by one of us (Z. S.) in Tel Amal, in the eastern plain of Esdraelon, which were at the time classified as a form of *Anopheles multicolor*.

On 28.xi.1940 Miss M. Hurwitz, the local malaria inspector in Tel Amal, collected *Anopheles* larvæ which drew her attention by their peculiar attitude in the water, i.e., the anterior part of their body was hanging down as in culicine larvæ. Some more larvæ were collected from the same locality in January 1941. From some of these adults were reared, which were sent to us for identification along with some preserved larvæ. The mosquitoes proved to be a new variety of *A. turkhudi*.

The material at our disposal consists of four larvæ, one adult male and one female in good condition and one female in bad condition. The two females which were caught in houses in January 1940 are not available at the moment. Unfortunately, no pupal skins were kept, nor were eggs obtained.

### DESCRIPTION.

#### ADULT: FEMALE.

*Head*: Vertex covered with white triangular scales in front and in middle and with similar dark scales at sides and posterior part of vertex. There is a short vertical tuft of narrow white scales and a few yellowish hairs. Palps black, with 3 narrow white rings at joints, terminal segment nearly all dark (Fig. 1).

*Thorax*: Middle part of mesonotum covered over its whole length with narrow sickle-shaped yellow scales which are absent on the sloping lateral part of the mesonotum.



*Wings* : (Fig. 2). Costa with only four white areas which are narrower than the black ones. Basal third of costa completely dark scaled.  $R_1$  (V.1) white at base with four black areas.  $R_{2+3}$  (V.2) mainly dark with narrow white areas near origin of  $R_{4+5}$  (V.3), at point of bifurcation, in middle of anterior branch and at tip of both branches.  $R_{4+5}$  (V.3) nearly all white, with a small dark spot near base and at tip. Media (V.4) nearly all dark except for narrow white areas round cross veins  $r-m$  and  $m-cu$ , at bifurcation and in middle of anterior branch. Cubitus (V.5) with dark spot near base and another at point of bifurcation covering both branches for a short distance. Anterior branch nearly all dark, except for spot opposite cross vein  $m-cu$ . Another light spot opposite bifurcation of media (V.4) and a few white scales at tip. Posterior branch white, except for small dark spot near tip. Analis (V.6) all dark, except for a few white scales in middle on anterior side of vein. Fringe with 5 to 6 white spots.

*Legs* : (Fig. 3). Dark except for knee, tip of tibia and inner surface of femora. In the hind femur, there is a well-defined white longitudinal stripe which ends a short distance before the tip. On the other femora the white stripe is less clearly defined.

*Abdomen* : Covered with hairs only.

#### ADULT: MALE.

Antennæ with mixed brown and white hair. Palps : (Fig. 4). Second segment all white except for a few dark scales at base. Complete white bands at juncture of 3rd and 4th, of 4th and 5th segments, and at tip. Club rather slender with long yellowish hairs. Thorax and legs as in female. Wings as in female, but with a small additional white spot at base of costa opposite humeral cross vein.

*Hypopygium* : (Fig. 5a). Theka with 10 to 11 leaflets increasing in size towards tip. The longest leaflet is about half as long as the theka. Harpagones with club-shaped lateral composite process, rather long apical spine, two external accessory hairs which are a third of the length of the apical spine and a very minute internal accessory hair; 4 spines at base of side piece of one side and 5 on that of the other (Fig. 5b), of which 3 are rather long and slightly curved distally, while the 4th is shorter and stouter.

We had only one female of *A. turkhudi* from India (Chakdara) for comparison, which had been kindly given to us by Prof. P. A. Buxton of the London School of Hygiene and Tropical Medicine. Our females differ from this specimen in the following details: there are two white spots on the basal third of the costa in the Indian specimen, one opposite the humeral cross vein and the other a bit more apically, while this area is completely dark in the Palestinian material. The base of  $R_1$  (V.1) is black in the Indian specimen and white in our specimens.  $R_{4+5}$  (V.3) is nearly all dark in the Indian specimen except for a few scattered white scales, while it is nearly all white in the Palestinian specimens. There is a well-defined white area in the middle of the analis in the Indian specimen, which is indicated by 4 to 5 white scales only on the anterior side of the vein in one of our females. Both our and the Indian specimens have narrow scales all over the



# Figures.



*Anopheles turkhudi* var. *telamali* var. nov.

1. Palps of female. 2. Wing of female. 3. Femur and tibia of hindleg of female.  
4. Palp of male. 5a. Hypopygium of male. 5b. Basal spines of left side of same.

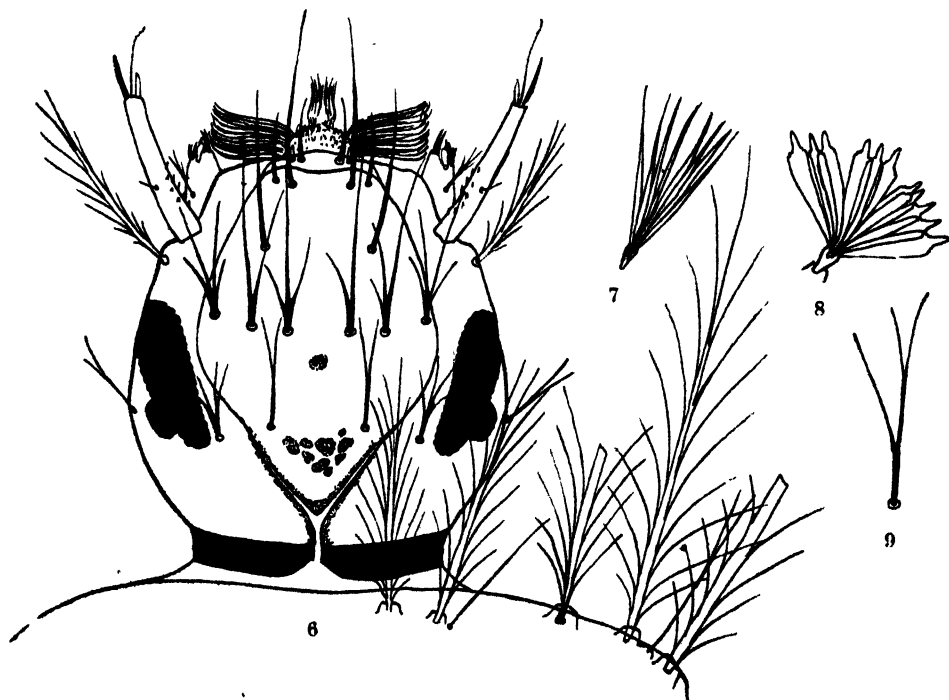


middle part of the mesonotum and not only in the anterior third of the mesonotum, as stated by Puri (1941). The male palps also differ apparently from those of Indian *A. turkhudi*, as Puri states that 'the club of the palpi is mainly dark, with such pale areas as are present ill defined and forming narrow lines only'. The male hypopygium is as figured by Christophers (1915), except that he gives the length of the leaflets on the theka as a quarter of the length of the theka. There are only 3 to 4 indicated in his figure while there are 10 to 11 in our specimen.

#### LARVA.

*Head* : (Fig. 6). Spots on vertex much reduced. Central spot very small or absent, lateral spots absent. Basal spot irregularly triangular formed by

#### Figures.



Larva of *Anopheles turkhudi* var. *telamali* var. nov.

6. Head of 4th stage larva. 7. Rudimentary palmate hair of abdominal segment 3. 8. Palmate hair of segment 5. 9. Hair No. 1 of segment 7.

several small spots. Mouth brushes narrow and projecting sideways as described by Iyengar (1930). Preclypeal hairs well developed. Outer anterior clypeal



hairs about half the length of the inner ones, all simple. Posterior clypeal hair simple and very long. Frontal hairs with very few (2 to 5) branches. Inner sutural hair bifid, outer sutural hair with 3 to 5 branches. Subantennal hair with about 12 branches. Antennal hair short and simple at about  $\frac{2}{3}$ ths of length of shaft. A few short spines at inner surface of antenna in basal part.

*Thorax* : Prothoracic submedian hairs, Nos. 1 and 2 long and feathered, No. 3 simple and fairly long. Metathoracic pleural hairs only 3 in number as stated by Puri.

*Abdomen* : Palmate hairs on segments 3 to 6 only. A rudimentary palmate hair (Fig. 7) on segment 3 and fully-developed darkly pigmented palmate hairs on segments 4 to 6 (Fig. 8). These palmate hairs have 11 to 13 leaflets with a well-marked shoulder and a short stumpy filament. There is no palmate hair on segment 7, but hair No. 1 is an ordinary hair with 2 to 4 branches (Fig. 9). In all other details the larva is exactly as described by Puri.

Length of larva 6 mm.

The main differences between the Palestinian larvæ and the Indian ones, as described by Puri (1931), are the bifid inner sutural hair which is simple in the Indian larvæ, the presence of a rudimentary palmate hair on segment 3 which is absent in the Indian larvæ, and the absence of the palmate hair on segment 7 on which there is a rudimentary palmate hair in the Indian larvæ. Only in one specimen among a large series of Indian larvæ of *A. turkhudi* examined by Puri, was a rudimentary palmate hair found on segment 3 on one side.

The *Anopheles* described above is more closely related to *A. turkhudi* than to any other species of the *Myzomyia* group with dark-tipped palpi. It differs, however, in several characters which are constant in our small series. These differences are very marked in the wing venation; but as we have not sufficient material for comparison and the wing venation is notoriously variable in this group, it is difficult to establish the status of our insect exactly before more material for comparison is available. However, the differences mentioned seem to justify the creation of a separate variety, and we propose the name *Anopheles turkhudi* var. *telamali* var. nov. Whether this status will remain valid after a more thorough study remains to be seen.

The larvæ were found in December in a seepage water originating from a fish breeding pond in Tel Amal and containing a rich surface vegetation of *Cladophora*. The temperature of the water was 22°C. They came to the surface for a few moments only and remained at the bottom most of the time. They exhibited the culicine attitude characteristic for *A. turkhudi* as described by Iyengar (*loc. cit.*) and Puri (1941), apparently owing to the absence of palmate hairs on the anterior part of the body. Only two adults were caught in houses among a great number of *A. sergenti*. Apparently it is not a domestic mosquito, and does not habitually enter houses.

The distribution of *A. turkhudi* and its varieties is a very wide one. It has been recorded from the whole western part of India as far south as Mysore. Shingarew (1928) records it from Samarkand, Christophers (1924) and Lindberg (1936) from



## 434 On A New Variety of *Anopheles turkhudi* from Palestine.

Persia (Kuchan Meshed and Shiraz). Salem (1938) records it from Sinai, and Lega, Raffaele and Canalis (1937) from Eritrea. Patton (1905) records *A. turkhudi* var. *azriki* from Aden Hinterland, and *A. flaviceps* Edwards from the Sudan may possibly also have to be included in this group (Edwards, 1921). The relationship of all these various forms remains to be studied in detail. One of us (O. T.) intends to do this and also to include related species and would be grateful for the loan of material of *A. turkhudi* and its varieties from different localities and for material of *A. hispaniola*, *broussesi*, *flaviceps* and *italicus*.

### REFERENCES.

- |   |  |
|---|--|
| CHRISTOPHERS, S. R. (1915) ..                   | .. The male genitalia of <i>Anopheles</i> . <i>Ind. J. Med. Res.</i> , <b>3</b> , p. 371.  |
| <i>Idem</i> (1924) ..                           | .. Provisional list and reference catalogue of the <i>Anophelini</i> . <i>Ind. Med. Res. Mem.</i> No. 3.                                       |
| EDWARDS, F. W. (1921) ..                        | .. Mosquito Notes II. <i>Bull. Ent. Res.</i> , <b>12</b> , 1, p. 69.   |
| IYENGAR, M. O. T. (1930) ..                     | .. The larva of <i>Anopheles turkhudi</i> . <i>Ind. J. Med. Res.</i> , <b>17</b> , p. 1189.  |
| LEGA, G., RAFFAELE, G., and CANALIS, A. (1937). | .. Missione dell'Istituto di Malariologia nell'Africa Orientale Italiana. <i>Riv. Malariol.</i> , <b>16</b> (1), 5, p. 325.                    |
| LINDBERG, K. (1936) ..                          | .. Le Paludisme dans l'Iran. <i>Ibid.</i> , <b>15</b> (1), 2, p. 132.  |
| MARTINI, E. (1931) ..                           | .. Culicidae in Lindner. <i>Flieg. Palaearkt. Reg.</i> , <b>11</b> and <b>12</b> , p. 321. Stuttgart.  |
| PATTON, W. S. (1905) ..                         | .. The Culicid fauna of the Aden Hinterland, their haunts and habits. <i>J. Bombay Nat. Hist. Soc.</i> , <b>16</b> , 4, p. 632.                |
| PURI, I. M. (1931) ..                           | .. Larvæ of anopheline mosquitoes, with full description of those of the Indian species. <i>Ind. Med. Res. Mem.</i> No. 21.                    |
| <i>Idem</i> (1941) ..                           | .. Synoptic table for the identification of anopheline mosquitoes of India. 3rd Ed. <i>Hlth. Bull.</i> No. 10. Manager of Publications, Delhi. |
| SALEM, H. H. (1938) ..                          | .. The mosquito fauna of Sinai Peninsula (Egypt) with a description of two new species. <i>Publ. Fac. Med. Egypt. Univ.</i> No. 16, Cairo.     |
| SHINGAREW, N. I. (1928) ..                      | .. Notes on Culicidae III (In Russian). <i>Russ. J. Trop. Med.</i> , <b>6</b> , 1, p. 47.  |
| STACKELBERG, A. (1927) ..                       | .. Culicidae of U.S.S.R. and adjoining countries (In Russian). <i>Tab. anal. Faune. U.S.S.R. Mus. Zool. Acad. Sci. Leningrad.</i>              |



## STUDIES ON MALARIA IN THE DELTAIC REGION OF BENGAL.

BY

M. O. T. IYENGAR, D.Sc.

*(Entomologist, Bengal Public Health Department).*

[May 18, 1942.]

Of the different topographical divisions comprising the Province of Bengal (Iyengar, 1929a ; 1938), the most extensive is the deltaic region, an alluvial tract which is essentially flat and devoid of surface undulations. The distribution of endemic malaria in this region differs markedly from that recorded in other parts of the Province and elsewhere. It has been observed that wet areas in the deltaic region\* are comparatively free from malaria, while dry areas show a high incidence of the disease (Iyengar, 1929b). This has been the experience not only of the present writer but of other observers as well. No convincing explanation has yet been put forward to explain this phenomenon.

Investigations recorded by Fry (1914), Bentley (1925), Schüffner (1931) and Iyengar (1928) indicate that the depth of the subsoil water table from the ground surface has an important bearing on the endemicity of malaria in the deltaic region. These observers noted that where the subsoil water level was high, malaria was practically absent, whereas areas with a low water table were highly endemic. Bentley (1925, Appendix I, pp. ix-x) states: 'Recent observations in Burdwan, Nadia, Jessore, Pabna and Murshidabad... have proved that... the areas in which the level of the subsoil water is specially low are far more malarious and unhealthy than those in which it approaches within 3 to 5 feet of the ground surface. In Burdwan, which is one of the worst districts for malaria and depopulation, the mean level of the subsoil water taken in twenty-eight wells in different parts of the district is 26 feet in the dry weather and 9 feet in the rains; whereas in healthy parts of Howrah, Dacca and Mymensingh, it varies from 3 to 5 feet in the dry weather and is level with the ground surface in the rains'. The same author (Bentley, 1928), commenting on the results of an investigation made by Iyengar (1928) in the district of 24-Parganas, states: 'The characteristics of the elevated zones are

---

\* In the present article the author discusses only the main part of the delta and excludes from consideration the estuarine zone, where conditions are different owing to the salinity of the soil and of the water collections.



comparative dryness of the villages, a low subsoil water level and a relatively scanty number of large-sized collections of water... In the low-lying areas, on the other hand, there is much accumulation of water in and around the villages, the subsoil water level is close to the surface and plentiful opportunities are offered for the raising of wet crops. In these two types of zones, the present survey (of Iyengar) has shown that the malaria prevalence is very different—while the more elevated tracts are highly endemic, the low-lying zones are nearly malaria-free'. Iyengar (1929a, p. 121) remarked: 'The nearness of the subsoil water in this deltaic tract corresponds with the non-malariousness of the locality'. In a later paper (Iyengar, 1929b), he recorded that in deltaic areas with a low subsoil water level the spleen rates were high (over 60 per cent), while in areas with a high water table they were low (under 9 per cent).

These observations were critically examined by the members of the Malaria Commission of the League of Nations who toured Bengal in 1929, and Dr. Schüffner, the President of the Commission, remarked: 'In the Province of Bengal... the conditions of malaria are exactly the opposite of those in the Punjab. In the delta of the Ganges and Brahmaputra, malaria is slight everywhere where the subsoil water table is very high. It is prevalent only where the table is at a lower level' (Schüffner, *loc. cit.*).

While these observations showed how in the deltaic region of Bengal malaria was closely associated with a low water table, the exact manner in which this factor influenced malaria endemicity was not clearly understood. Iyengar (1929b) observed that, in dry areas of the delta, a high incidence of *Anopheles varuna* Iyengar occurred during the period of the year when atmospheric conditions of humidity and temperature were favourable for the transmission of malaria infection, and that in wet villages the incidence of this species did not reach a high level till towards the close of the transmission season. Iyengar, believing that *A. varuna* was the principal vector in the area, thought that the high incidence of malaria in the dry villages was due to the fact that the prevalence of this species coincided with the period when conditions of atmospheric humidity and temperature were favourable for the transmission of malaria infection; whereas in the wet villages the absence of malaria was ascribed to the fact that the period of high prevalence of *A. varuna* did not coincide with the season favourable for malaria transmission. Later researches have shown that *A. philippinensis* Ludlow, and not *A. varuna*, is the primary vector of malaria in deltaic Bengal. Consequently, the variations in the seasonal incidence of *A. varuna* in the different areas would not explain the differences in malarial endemicity.

#### STUDIES IN THE DELTAIC AREA.

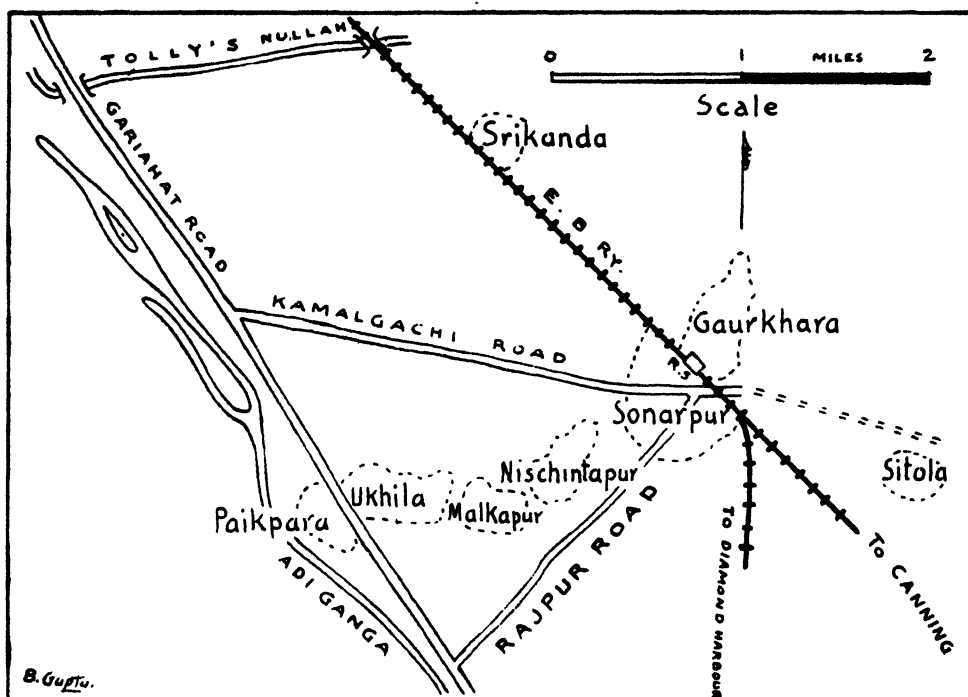
Observations carried out in a rural area in the district of 24-Parganas seem to throw some light on the factors that influence malarial endemicity. Near Sonarpur there is a dry area on the west and a wet area on the east, about three to four miles apart. The western area is on a slightly higher elevation than the eastern, but the difference in levels is so small (10 to 15 feet) that it is scarcely evident to the eye. The average rainfall is about 65 inches. The western area is better drained in



comparison with the eastern, which appears to have no adequate drainage facilities. The incidence of malaria in the two areas differs considerably. In the villages on the west the spleen rates are higher than 40 per cent, sometimes as high as 70 per cent, while in the eastern area corresponding figures are from 0 to 3 per cent. In those situated in the intervening area, the rates tend to diminish as one approaches the low-lying area on the east. No obvious differences are noticeable in the two areas to account for this remarkable variation in malarial endemicity, since the composition of the population, the physical features of the land, the rainfall and the atmospheric temperature and humidity conditions are more or less similar. The only noticeable difference is that the subsoil water level is close to the ground surface in the eastern area, while in the western area it is considerably lower.

Figure 1.

Sketch map showing the villages mentioned in the paper.



In order to study the factors that determine malarial endemicity in this region, eight villages situated on a strip of land extending from the western to the eastern area were selected for observation, namely, Ukhila and Paikpara on the west, Sitola and Srikanda on the east, and Malkapur, Nischintapur, Sonarpur and Gaurkhara in the intervening area (Fig. 1). These villages are practically contiguous, the distance from Paikpara to Sitola being less than four miles. Sitola and Srikanda are situated on open country, with few large trees and no undergrowth. There are



extensive ricefields near them, and during the rainy season these are covered with water for about two months. The vicinity of the other six villages mentioned is not subject to flooding of this kind at any time of the year. Paikpara and Malkapur have numerous large trees and much rank undergrowth, locally known as 'jungle'. Sonarpur and Nischintapur are largely shaded by trees and undergrowth, but the vegetation is not as dense as at Paikpara or Malkapur. Ukhila is situated on open land, with few large trees and practically no 'jungle'. The mosquito breeding places in this area are stagnant water collections such as ponds, ditches and earthen drains which hold rainwater. All the villages possess many ponds and ditches which serve as sources of domestic water supply and sometimes for rearing edible fish. The majority of the ponds are overgrown with aquatic vegetation and are potential breeding places of mosquitoes. There is a slight variation in the elevation of the different villages mentioned, Ukhila being the highest, followed by Paikpara, Malkapur, Nischintapur, Sonarpur, Gaurkhara, Srikanda and Sitola in that order. The subsoil water table is highest in Sitola, where it is close to the ground surface, and lowest in Ukhila (*vide infra*).

The villages selected for study show marked difference in regard to the incidence of malaria as judged by the spleen rates. All available children (under 12 years of age) in the eight villages were examined for splenic enlargement several times every year during the period 1929-1937 (Table I).

TABLE I.  
Average spleen rates for the period 1929-1937.

Village.	Spleen rate, per cent.
Sitola ..	0.00
Srikanda ..	0.02
Gaurkhara ..	2.28
Sonarpur ..	8.06
Nischintapur ..	17.97
Malkapur ..	20.80
Paikpara ..	28.05
Ukhila ..	43.07

#### ANOPHELINE FAUNA.

The following species of *Anopheles* occur in this area:—

<i>A. subpictus</i> Grassi.	<i>A. philippinensis</i> Ludlow.
<i>A. vagus</i> Dönitz.	<i>A. aconitus</i> Dönitz.
<i>A. barbirostris</i> Wulp.	<i>A. varuna</i> Iyengar.
<i>A. hyrcanus</i> var. <i>nigerrimus</i> Giles.	<i>A. tessellatus</i> Theob.
<i>A. annularis</i> Wulp.	<i>A. culicifacies</i> Giles.
<i>A. ramsayi</i> Covell.	<i>A. theobaldi</i> Giles.
<i>A. pallidus</i> Theob.	



Four of these species, namely, *A. tessellatus*, *A. culicifacies*, *A. pallidus* and *A. theobaldi*, are very rare in this area and only stray specimens of these species have been observed. The other nine species are fairly common.

Dissections of adult mosquitoes caught in Ukhila and other endemic villages in the neighbourhood showed an infectivity rate of 15.5 per cent in *A. philippinensis* and 0.7 per cent in *A. varuna* (Iyengar, 1940, p. 121). The sporozoite rate in *A. philippinensis* was 12.7 per cent, while that of *A. varuna* was nil. From the high infection rate observed in *A. philippinensis* it is evident that this species is the primary malaria vector in the area.

*A. philippinensis* has also been found infected in other parts of deltaic Bengal. Sur (1928) and Bose (1932) found it infected in Nadia district and Sur (1934) in Burdwan and Faridpur districts. Iyengar (1939) recorded a high infectivity rate in this species in Hooghly, Nadia and Rangpur districts, and again in Faridpur, Midnapur, 24-Parganas, Rajshahi and Tippera (Iyengar, 1940). Natural infection in *A. philippinensis* has since been observed in the districts of Mymensingh, Jessore and Nadia (author's unpublished records). The finding of natural infection among *A. philippinensis* in the malarious tracts of deltaic Bengal and the high infection rates frequently recorded, indicate that *A. philippinensis* is the primary vector of malaria in deltaic Bengal.

All collections of water in the villages under observation were examined regularly twice a month. The results of larval collections in respect of *A. philippinensis* during the period 1929-1937 are presented in Table II.

TABLE II.

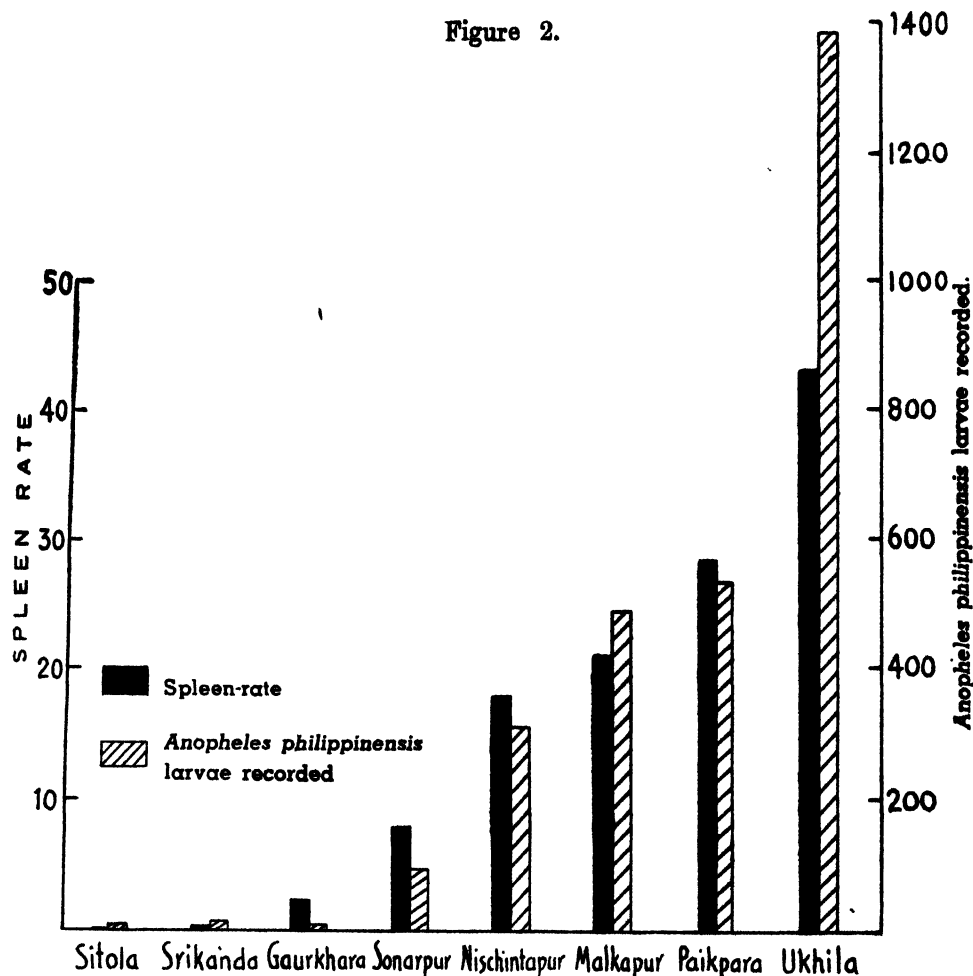
*Larval collections of A. philippinensis, 1929-1937.*

Village.	Number of Anopheles larvæ collected.	Number of <i>A. philippinensis</i> larvæ collected.	Percentage of <i>A. philippinensis</i> .
Sitola .. ..	51,486	4	0.008
Srikanda .. ..	56,218	8	0.014
Gaurkhara .. ..	82,486	10	0.012
Sonarpur .. ..	129,618	94	0.073
Nisohintapur .. ..	84,500	311	0.366
Malkapur .. ..	82,366	490	0.595
Paikpara .. ..	42,450	529	1.246
Ukhila .. ..	130,500	1,383	1.060



The prevalence of *A. philippinensis* is lowest in Sitola, where out of about 51,500 larvæ collected only 4 specimens were of this species. The number of specimens of *A. philippinensis* recorded from the different villages increases progressively in the order given in Table II. When the figures relating to the spleen rates (Table I) and the incidence of *A. philippinensis* are compared, a direct variation is apparent (Fig. 2). Statistical examination shows a strong positive correlation between

Figure 2.



the two sets of figures. The co-efficient of correlation between the percentage incidence of *A. philippinensis* and the spleen rate is  $+0.92$  and that between the total number of *A. philippinensis* larvæ and the spleen rate is  $+0.96$ . Both these co-efficients of correlation are significant. These observations furnish evidence to show that the relative prevalence of *A. philippinensis* largely determines the malarial endemicity of the village.



As mentioned above, *A. varuna* was also found infected in this area. This species has also been found infected in Hooghly district (Iyengar, 1928, p. 16) and in Howrah district (Roy, 1939). In order to ascertain whether this species plays any part in the determination of the local malarial endemicity, the relevant data are presented for statistical analysis in Table III.

TABLE III.

*Larval collections of A. varuna, 1929-1937.*

Village.	Number of Anopheles larvæ collected.	Number of <i>A. varuna</i> larvæ collected.	Percentage of <i>A. varuna</i> .
Sitola .. ..	51,486	5,102	9.91
Srikanda .. ..	56,218	4,548	8.09
Gaurkhara .. ..	82,486	9,404	11.40
Sonarpur .. ..	129,618	19,784	15.26
Nischintapur .. ..	84,500	13,421	15.88
Malkapur .. ..	82,366	20,564	24.97
Paikpara .. ..	42,450	4,276	10.07
Ukhila .. ..	130,500	20,676	15.84

The incidence of *A. varuna* does not show any constant relation with the corresponding spleen rates (Table I). The co-efficient of correlation between the percentage incidence of *A. varuna* and the spleen rate is + 0.42, whilst that between the total number of *A. varuna* larvæ and the spleen rate is + 0.49. Neither of these co-efficients of correlation is statistically significant. This observation shows little relation between the incidence of *A. varuna* and the malarial endemicity of the village. Though this species may be a potential vector of malaria and has been found infected in some places, it does not appear to play any important part in this area in determining local malarial endemicity.

#### SEASONAL INCIDENCE OF *A. PHILIPPINENSIS*.

Iyengar (1932) showed that the main breeding season of this species was from July to October. The data for the period 1929-1937 have been analysed to show



the incidence of *A. philippinensis* larvæ during different months (Table IV). It will be observed that the season of maximum prevalence of the larvæ of this species is from July to October, which confirms the finding previously recorded.

TABLE IV.

*Average monthly larval collections of A. philippinensis, 1929-1937.*

Month.	Number of Anopheles larvæ collected.	Number of <i>A. philippinensis</i> larvæ collected.	Percentage of <i>A. philippinensis</i> .
January .. ..	52,112	88	0·17
February .. ..	44,523	45	0·10
March .. ..	46,616	45	0·10
April .. ..	56,070	171	0·30
May .. ..	60,863	213	0·35
June .. ..	66,014	262	0·40
July .. ..	58,573	512	0·87
August .. ..	67,533	455	0·67
September .. ..	67,716	446	0·66
October .. ..	36,209	280	0·77
November .. ..	50,504	155	0·31
December .. ..	52,893	157	0·30

SUBSOIL WATER LEVEL IN RELATION TO PREVALENCE OF  
*A. PHILIPPINENSIS.*

The variations in the prevalence of *A. philippinensis* in the different villages (Table III) do not appear to be due to any differences in the facilities offered for the



breeding of this mosquito. Ponds are the usual breeding places of *A. philippinensis* and these exist in all the eight villages studied. There do not appear to be any marked differences either in the number or the type of ponds in the different villages to account for the variations in the prevalence of this species.

On the other hand, if we examine the data relating to the subsoil water table recorded during the breeding season of *A. philippinensis*, there appears to be a close relation between the distance of the water table from the ground surface and the numerical prevalence of this mosquito (Table V).

TABLE V.

*Subsoil water level during the wet season (August).*

Village.	Distance of sub-soil water from ground level (in inches).
Sitola .. ..	0
Srikanda .. ..	17
Gaurkhara .. ..	21
Sonarpur .. ..	34
Nischintapur .. ..	36
Malkapur .. ..	38
Paikpara .. ..	51
Ukhila .. ..	62

A comparison of Table II with Table V shows a close relationship between the level of the subsoil water table and the prevalence of *A. philippinensis*. The co-efficient of correlation between the percentage incidence of *A. philippinensis* and the depth of the water table below the ground surface is + 0.86, and that between the total number of *A. philippinensis* larvæ collected and the depth of the water table is + 0.85. Both these co-efficients of correlation are statistically significant.



While it is obvious that in the deltaic area, the level of the subsoil water during the breeding season is a measure of the prevalence of *A. philippinensis*, the exact manner in which this factor operates remains unexplained.\*

The findings above recorded appear to open up a new field in the problem of malaria control in deltaic Bengal. In order to combat malaria in this region, measures should be directed primarily against *A. philippinensis*, the important vector of malaria in the area. The feasibility of species control is, however, rendered difficult by the factor that the preferential breeding places of this mosquito do not differ essentially from those of other species of *Anopheles* occurring in the same area.

Nevertheless, since a high subsoil water table has been shown to be inimical to its breeding, it should be possible to control it by raising its level during the breeding season. This might be effected by the introduction and conservation of water.† It is suggested that this hypothesis should be put to test by experimentally raising the water table in a part of the deltaic area and studying the effect of such a measure on the local incidence of malaria.

It is possible that malaria workers, who have no first-hand knowledge of the conditions prevailing in deltaic Bengal, may view this suggestion with disfavour. Since the introduction of irrigation in certain parts of India has been associated with an increase in the incidence of malaria, they may apprehend that the raising of the water table in deltaic Bengal may increase the facilities for mosquito breeding and thus render the area more malarious. It should be pointed out, however, that such areas as have suffered an increase of malaria consequent on the introduction of irrigation are non-deltaic and dry, and cannot, therefore, be taken as standards for judging the conditions in flat deltaic regions which are normally wet and have a high subsoil water table. Considering the beneficial effect of a high water table on the incidence of malaria and its inhibitory influence on the prevalence of *A. philippinensis*, the primary vector of malaria in the region, it is unlikely that the raising of the water table will have anything but beneficial results.‡

Epidemiological observations support the view that the raising of the water table in the deltaic region of Bengal would tend to lower the incidence of malaria. Several parts of this region where stormwater is impounded through lack of proper drainage facilities and where there is a constant demand for drainage measures, are almost invariably free from malaria. On the other hand, areas which suffer from drought and where the natural collections of water dry up rapidly because of a low water table, are generally associated with hyperendemic malaria. It has also been observed that during years of abnormally heavy rainfall the incidence of

---

\* It would appear that the breeding of *A. philippinensis* is associated with the presence of certain types of algal plankton, and that the lack of soil aeration consequent on a high subsoil water level inhibits the development of the type of plankton favourable for larvae of *A. philippinensis*. This aspect of the problem requires further investigation.

† Irrigation would be one of the measures for raising the water table.

‡ Bentley (1925, Appendix IV, p. xliv) stated that the introduction of an added supply of water into malarious tracts of deltaic Bengal had led to an improvement in health.



malaria is low, whilst years of deficient rainfall are associated with an increased prevalence of the disease. In all the instances mentioned above, it would appear that the factor that determines the prevalence of malaria is the level of the subsoil water table which is influenced by variations in rainfall, abundance or lack of water and the presence or absence of drainage facilities.

### SUMMARY.

1. In the deltaic region of Bengal, contrary to conditions prevailing in other regions, areas with a high subsoil water level during the wet season are comparatively free from malaria, while those with a low subsoil water level show a high incidence of the disease. The factors that would account for the malariousness of dry areas and the healthiness of wet areas have hitherto not been clearly understood.

2. In order to study the factors that determine malarial endemicity in this region, a series of observations were carried out in a group of villages with different spleen rates. The local malaria vector was shown to be *A. philippinensis*. This species has been found infected in different parts of deltaic Bengal, often with high infection rates.

3. A high prevalence of *A. philippinensis* is associated with a high malarial incidence, and a low prevalence of this mosquito with a low malarial incidence. The prevalence of *A. philippinensis* shows a significant positive correlation with the spleen rate.

4. The main breeding season of *A. philippinensis* is from July to October. The depth of the water table below the ground surface during the breeding season of *A. philippinensis* appears to have a marked influence on the prevalence of this species. In villages with a low water table the density of *A. philippinensis* was high. In villages where the water table was close to the ground surface the incidence of this mosquito was very low. A significant positive correlation was observed between the density of *A. philippinensis* and the distance of the water table from the ground level during the wet season.

### ACKNOWLEDGMENTS.

I am much indebted to Mr. S. Swaroop, M.A., Statistician, Public Health Department, Office of the Director-General, Indian Medical Service, for working out the statistical correlations mentioned in this article.

### REFERENCES.

- |                       |    |    |  |
|-----------------------|----|----|--|
| BENTLEY, C. A. (1925) | .. | .. | Malaria and Agriculture in Bengal. Bengal Seortt. Book Depot, Calcutta.  |
| <i>Idem</i> (1928)    | .. | .. | Preface to IYENGAR (1928).   |
| BOSE, K. (1932)       | .. | .. | <i>Rec. Mal. Surv. Ind.</i> , 3, 1, pp. 161-162.                         |
| FRY, A. B. (1914)     | .. | .. | Second report on malaria in Bengal. Bengal Seortt. Book Depot, Calcutta. |



- |                             |    |   |
|-----------------------------|----|---|
| IYENGAR, M. O. T. (1928) .. | .. | Report on the malaria survey of the environs of Calcutta.<br>Bengal Secrty. Book Depot, Calcutta. |
| <i>Idem</i> (1929a) ..      | .. | <i>Trans. F. E. A. T. M. 7th Congress</i> , <b>3</b> , pp. 116-126.                               |
| <i>Idem</i> (1929b) ..      | .. | <i>Ibid.</i> , <b>2</b> , pp. 684-694.  |
| <i>Idem</i> (1932) ..       | .. | <i>Ind. J. Med. Res.</i> , <b>19</b> , 3, pp. 917-939.  |
| <i>Idem</i> (1938) ..       | .. | <i>Proc. Nat. Inst. Sci. India</i> , <b>4</b> , 2, pp. 181-184.                                   |
| <i>Idem</i> (1939) ..       | .. | <i>J. Mal. Inst. Ind.</i> , <b>2</b> , 1, pp. 105-109.  |
| <i>Idem</i> (1940) ..       | .. | <i>Ibid.</i> , <b>3</b> , 1, pp. 115-123.   |
| ROY, D. N. (1939) ..        | .. | <i>Ibid.</i> , <b>2</b> , 3, pp. 239-242.   |
| SCHUFFNER, W. (1931) ..     | .. | <i>Rec. Mal. Surv. Ind.</i> , <b>2</b> , 3, pp. 337-347.  |
| SUR, P. (1928) ..           | .. | <i>Ind. J. Med. Res.</i> , <b>16</b> , 1, pp. 45-47.  |
| <i>Idem</i> (1934) ..       | .. | Private communication.  |



## A NEW VARIATION OF *ANOPHELES GAMBIAE*.

BY

CAPTAIN B. DE BURCA, I.M.S.,

AND

LABORATORY ASSISTANT MOHD. YUSAF.

[May 21, 1942.]

A NUMBER of mosquitoes were bred out of a batch of larvæ collected by one of us (M. Y.) from a rainwater pool in an air-raid shelter at Massawa, Eritrea, in December 1941. With the exception of a single female specimen\*, which was somewhat pale in colour and did not conform to any species previously described, all the mosquitoes hatched out were either *A. gambiae* or *A. dthali*. No other species of Anopheles was encountered during the malaria season in this locality.

At first sight, the pale ornamentation of the specimen alluded to above led us to suspect that it might belong to a species hitherto undescribed, but the presence of speckling on the legs and the close resemblance of the pharyngeal armature to that of *A. gambiae* suggest that it is more probably a very pale form of the latter species. It does not correspond, however, to any previously described forms of 'albinoid *gambiae*' (Evans, 1938).

Unfortunately, the larval and pupal moults were not recovered. A brief description of the female specimen is as follows :—

*Adult*.—A medium sized mosquito with brown body, wings mainly pale, and legs with conspicuous pale markings.

*Head*.—Pale head scales tinged with yellow on the posterior part of vertex ; frontal tuft well developed. Antennæ with a few pale scales on the torus and on the inner surface of the first two flagellar segments. Palpi with only two pale bands. There is a very broad distal pale band extending from tip to middle of third segment and a narrow pale band at distal end of second segment. Semi-erect scales on dorsal surface of first and part of second. Proboscis dark, with pale labella.

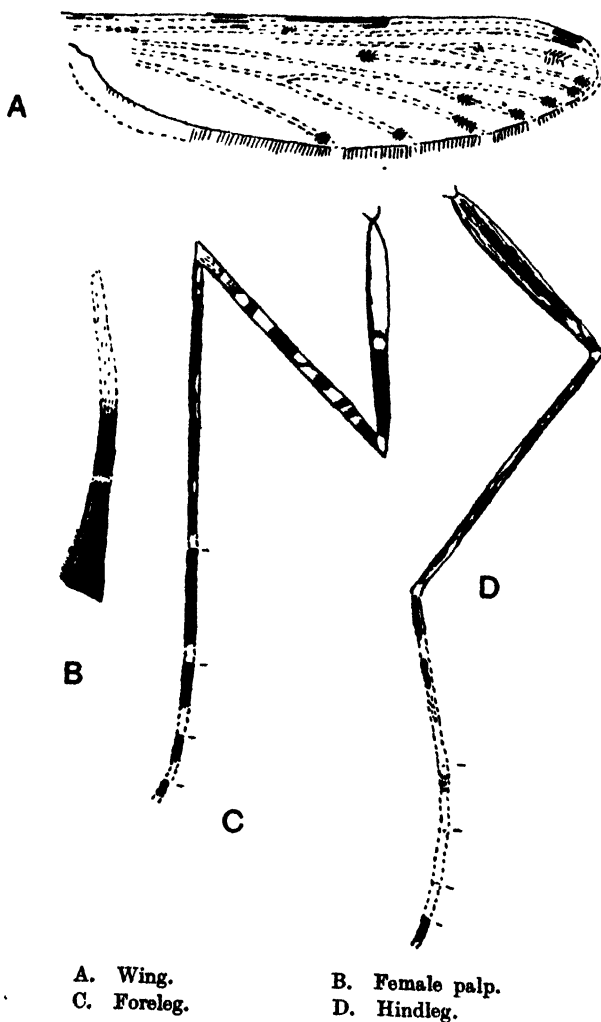
---

\* It is proposed to deposit the specimen in the British Museum when circumstances permit.



*A New Variation of Anopheles gambiæ.*

*Pharynx*.—Closely resembles that of *A. gambiæ*. Sixteen pairs of rods and cones. The latter have deep roots and long filaments bearing a few spicules. Pharyngeal ridges with long hair-like processes.



A. Wing.  
C. Foreleg.

B. Female palp.  
D. Hindleg.

*Thorax*.—Integument of mesonotum dark-brown with narrow median and submedian dark stripes. The submedian stripes are somewhat widened about the middle. Median area clothed with bristles and yellowish pointed scales which are more numerous on the anterior part of mesonotum. Anterior promontory with a cluster of pale scales. Scattered pale scales present on fossæ.



*Abdomen*.—Integument light brown to black. Clothed thinly with yellowish-brown hairs. A few pale scales present on distal half of eighth tergite.

*Forelegs*.—Femora and tibiae with diffuse speckling. Tibiae of the two sides show marked variations in their ornamentation; on one there is irregular coarse speckling, while the other is mainly pale with a dark line along the anterior surface. First tarsal segment with two or three dark bands on proximal  $\frac{2}{3}$ ; distal third pale; the second with only a few scattered dark scales on proximal half; third and fourth entirely pale; the fifth is dark.

*Mid- and hindlegs*.—Proximal half of femora pale with a dark longitudinal line anteriorly; distal half with a few irregular pale markings. Tibiae with coarse pale speckling. First tarsal segment mainly dark with a few irregular pale markings and a pale apical band, the second is all dark with a broad apical pale band, the third and fourth pale on distal  $\frac{1}{3}$ , and the fifth all dark with a few pale scales distally.

*Wings*.—Mainly pale with a few dark spots. A pair of very narrow dark spots lie near the base of the costa, but there are no corresponding areas on first longitudinal vein. The inner costal spot not very broad; the middle dark part represented on vein 1 by only a few dark scales. The only other spot on the costa and the first longitudinal vein lies near distal end of costa. Vein 2 bears a few dark scales at the base of its anterior branch. A few dark scales also present at the distal ends of both its branches. Vein 3 mainly pale with narrow dark spots at both ends. Vein 4 also mainly pale with only narrow dark spots at the distal ends of its branches and a few dark scales near the base of the lower branch. Veins 5 and 6 with narrow black spots near the apices. Pale fringe spots present at the ends of all the veins.

#### ACKNOWLEDGMENTS.

We are indebted to Dr. I. M. Puri, Entomologist, Malaria Institute of India, and Mr. D. J. Lewis, Entomologist, Sudan Medical Service, for suggestions, and to the latter for examining the pharyngeal armature.

#### REFERENCE.

- EVANS, A. M. (1938) .. .. Mosquitoes of the Ethiopian Region, 2, p. 305. Brit. Museum Pubn., London.







## NOTES ON THE MOSQUITO FAUNA OF ROT-HOLES IN TREES AND BAMBOO STUMPS IN CEYLON.

BY

D. P. WIJESUNDARA

(Division of Medical Entomology, Department of Medical and Sanitary  
Services, Ceylon).

[July 29, 1942.]

COMPARATIVELY little information on the mosquitoes breeding in rot-holes in trees and bamboos in Ceylon is available. James (1914), in the course of a mosquito survey of Colombo, recorded the following species: *Stegomyia fasciata* [= *Aedes (Stegomyia) aegypti*], *S. scutellaris* Theo. nec Walk. [= *Aedes (Stegomyia) albopictus*], *S. gubernatoris* [= *Aedes (Finlaya) gubernatoris*], *S. micropterus* James nec Giles [= *Aedes (Diceromyia) reginae*] and *Armigeres obturbans*. He stated that the great majority of the larvæ found in the tree-holes were those of *Aedes gubernatoris* and *Aedes reginae*, and in his analyses of breeding places gave tree-holes and bamboos (taken together) as forming on an average 1.06 per cent of actual breeding places of *Aedes aegypti* in Colombo, 8.5 per cent of those of *Aedes albopictus*, and 7.5 per cent of those of *Armigeres obturbans*. Senior White (1920), working at Matale (northern hills, altitude approximately 1,200 ft.), recorded *A. gubernatoris*, *C. brevipalpis*, *O. anopheloides*, *Tororhynchites immisericors* (= *Megarhinus splendens*), *A. obturbans*, *Heizmannia greeni* and *Rachionotomyia* (= *Tripteroides*) *aranoides*. Carter (1925) noted that *Anopheles annandalei* (recorded by James as *A. asiaticus*) belonged to a group of species breeding in tree-holes and bamboos. With the exception of the last-named species, all those mentioned above have been found during the present investigations. *A. annandalei*, however, would seem to be very rare and the only record since James is that of a solitary male caught in a house at Koslanda (south-eastern hills, altitude 2,250 ft.), in March 1941.



## 452 *The Mosquito Fauna of Rot-Holes in Trees and Bamboo Stumps.*

The observations noted in this paper were carried out during the period October 1940 to February 1942. In the first series the observations were made from 15.x.40 to 25.xi.41 on samples of larvæ collected weekly from an accumulation of water in the fork of a tree (*Peltophorum ferrugineum*) situated near the Colombo laboratory; in the second series from early November 1940 to February 1942, on larvæ collected from numerous rot-holes in trees and bamboo stumps situated chiefly in the hill districts (elevations of from 450 ft. to 3,300 ft.) of Ceylon.

### TREE-HOLE, COLOMBO.

As indicated above this breeding site was in the fork of a tree; it was situated about 5 feet from the ground and measured approximately 6 inches  $\times$  7 inches  $\times$  9 inches deep; its capacity was approximately 3,500 c.c. The water present was usually pale brown in colour, but during, and for a short time after, heavy rain it became clear; except on one occasion (in June 1941), no pronounced odour was observed. No water was present in the fork during the periods 17.xii.40 to 24.xii.40 and 28.i.41 to 15.iv.41. The debris at the bottom consisted chiefly of rotting leaves and twigs together with pieces of glass from broken bottles and road metal introduced by labourers. Throughout the period of observation the pH of the water ranged from 6.8 to 7.8 with a higher reading of 8.4 for one sample collected in the middle of May 1941.

Except at the first examination on 15.x.40 when the greater part of the water contents (from which 758 mosquitoes were reared) was removed, the practice was to take only about one-third of the water present in the fork each week to the laboratory where the larvæ and pupæ collected were allowed to complete their development in dishes to which powdered prawns and ox-liver had been added. The numbers of adult mosquitoes so obtained averaged 148 per sample (44 samples) and varied from 33 to 307; the number of adults reared from each sample from 22.x.40 to 25.xi.41 is shown in association with rainfall in the Graph.

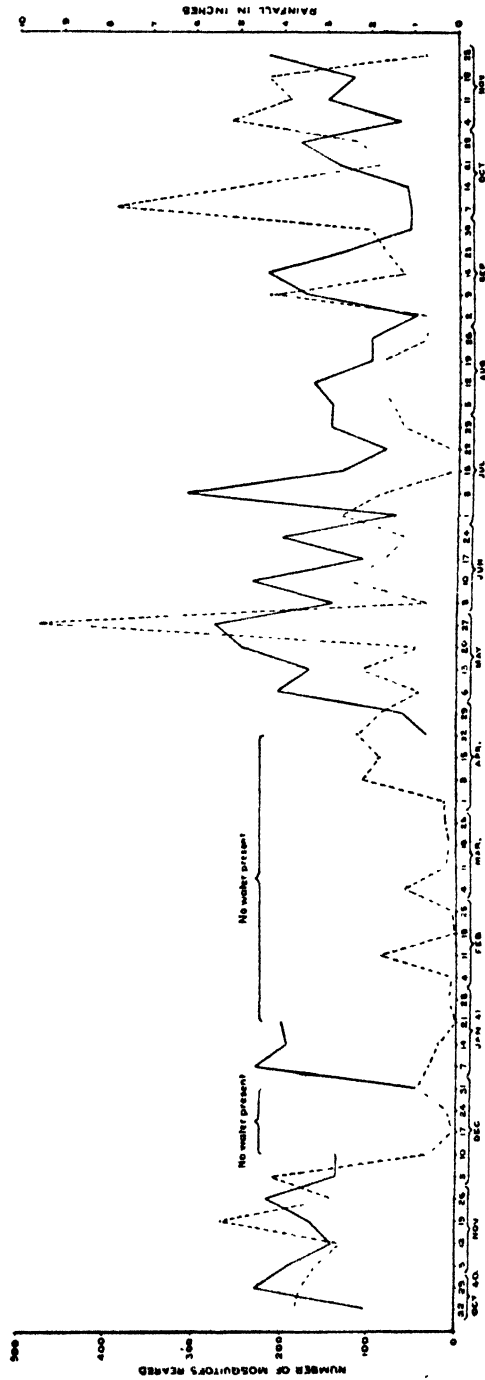
These findings suggest that the output of mosquitoes from such inconspicuous and strictly confined breeding places as rot-holes may be very considerable. As long ago as 1901, Green drew attention to this fact and made an interesting computation of the output of mosquitoes from stumps of the Giant Bamboo (*Dendrocalamus giganteus*) present in the Botanic Gardens at Peradeniya: 'By counting the number of larvæ actually present in a certain number of stumps, and the number of stumps then holding water in the several clumps of giant bamboos, it was estimated that there existed at the time 57,000 larvæ within the area of the Gardens. A simple calculation allowing for the time occupied in development and the period during which water was likely to remain in the stumps, resulted in a total of 912,000 mosquitoes bred in a year from this one source'. In the present instance, it is believed that the average output per week during approximately 9 months of the year from the single small hole under observation was probably not less than 400 to 500 mosquitoes.



GRAPH.

Breeding of tree-hole mosquitoes, Colombo : 22.x.40 to 25.xi.41.

— Number of Mosquitoes.  
- - - - Rain fall





## 454 *The Mosquito Fauna of Rot-Holes in Trees and Bamboo Stumps.*

In all 7,300 mosquitoes were reared in the laboratory from the larvæ and pupæ collected in the samples. Twelve species of culicines were represented, their numerical distribution being :—

<i>Culex (Neoculex) brevipalpis</i> (Giles)	..	3,524	(48.3 per cent.)
<i>Aedes (Stegomyia) albopictus</i> (Skuse)	..	1,797	(24.6 „ )
<i>Aedes (Finlaya) gubernatoris</i> (Giles)	..	1,688	(23.1 „ )
<i>Culex fatigans</i> Wied.	..	199	
<i>Aedes (Stegomyia) ægypti</i> (Linn.)	..	39	
<i>Armigeres obturbans</i> (Walk.)	..	22	
<i>Aedes (Diceromyia) regina</i> Edw.	..	21	
<i>Megarhinus splendens</i> (Wied.)	..	5	
<i>Culex vishnui</i> Theo.	..	2	
<i>Culex fuscocephalus</i> Theo.	..	1	
<i>Aedes (Finlaya) aureostriatus</i>			
var. <i>kanaranus</i> Barr.	..	1	
<i>Aedes (Stegomyia) vittatus</i> (Bigot)	..	1	

The first three species named were overwhelmingly predominant ; *C. brevipalpis* was present in every sample, *A. albopictus* in all except two samples, and *A. gubernatoris* in all except five samples. *C. fatigans* was found rarely, the majority of the adults reared being obtained from the first sample examined (15.x.40). *A. (S.) ægypti* occurred only occasionally (in 8 of 45 samples) and in small numbers.

On three occasions during the dry periods (in December 1940 and in January and February 1941), samples of moist debris were removed from the bottom of the fork and immersed in water at the laboratory. Larvæ appeared in the experimental jars some hours after immersion, and a total of 102 mosquitoes subsequently emerged ; these were *C. (N.) brevipalpis* (72 imagines), *A. (S.) albopictus* (22), *A. (F.) gubernatoris* (7), and *A. (S.) ægypti* (1). Egg-rafts of *C. (N.) brevipalpis* were rounded or broadly oval in shape and consisted of from 61 to 104 eggs (7 counts).

### TREE-HOLES AND BAMBOO STUMPS, HILL-COUNTRY DISTRICTS.

Collections of mosquito larvæ from rot-holes in trees and in bamboo stumps were made as opportunity allowed by the field assistants of the Division. The larvæ were sent to Colombo and the adults reared from them in the laboratory. The collections were made in the following localities : Kandy district (1,500 ft. to 1,800 ft.), Matale district (northern hill zone, 450 ft. to 1,200 ft.), Ratnapura district (western hills, 480 ft. to 1,700 ft.), and Badulla district (eastern hills, 2,200 ft. to 3,500 ft.).

Twenty-nine samples from trees of various species and twenty-three samples from stumps of the giant bamboo were received and examined. A total of 1,420 mosquitoes representing 19 species were reared from water obtained from the



tree-holes, and 1,352 representing 17 species from water from the bamboo stumps. The distribution of species in each group is given in the Table.

TABLE.

Species.	TREE-HOLES.		BAMBOO STUMPS.	
	Number.	Per cent.	Number.	Per cent.
<i>Aedes (S.) albopictus</i> (Skuse) .. ..	722	50·7	775	57·3
<i>Armigeres obturbans</i> (Walk.) .. ..	275	19·3	144	10·6
<i>Culex (Lophoceratomyia) uniformis</i> Theo. ..	117	8·2	235	17·4
<i>Aedes (F.) macdougalli</i> Edw. .. ..	108	7·6	3	} less than 1·0
<i>Aedes (S.) vittatus</i> (Bigot) .. ..	80	5·6	2	
<i>Aedes (F.) gubernatoris</i> (Giles) .. ..	36	2·5	2	
<i>Heizmannia greeni</i> (Theo.) .. ..	20	1·4	Nil	Nil
<i>Culex (N.) brevipalpis</i> (Giles) .. ..	17	1·2	50	3·7
<i>Tripteroides aranoioides</i> (Theo.) .. ..	3	} less than 1·0	47	3·5
<i>Aedes (S.) scutellaris</i> (Walk.) .. ..	5		33	2·4
<i>Megarhinus splendens</i> (Wied.) .. ..	8		25	1·8
Other species .. ..	29	2·0	36	2·7

'Other species' included: *A. (S.) aegypti*, *A. (F.) aureostriatus* var. *greeni*, and var. *kanaranus*, *A. (S.) novalbopictus* (tree-holes only), *C. fatigans*, *C. (Lutzia) vorax*, *C. (Lophoceratomyia) minutissimus* (bamboos only), *C. (Culiciomyia) shebbearei* (tree-holes only), *O. anopheloides* var. *maculata*, *Armigeres (Leicesteria) omissus* (bamboos only), and *Anopheles leucosphyrus*.

*A. (S.) scutellaris* (Walk. nec Theo.) and *A. (S.) novalbopictus* are recorded for the first time from Ceylon; *H. greeni*, *A. (F.) aureostriatus* var. *greeni* and var. *kanaranus*, and *A. (L.) omissus* have been rarely recorded previously. *C. (N.) brevipalpis* and *A. (F.) gubernatoris*, two of the predominant species in Colombo, were relatively scanty in the tree-holes examined in the hill districts, while *C. (L.) uniformis* was found only in the latter. *A. (S.) aegypti* was seldom present. *Anopheles leucosphyrus* recorded from bamboo stumps by Hacker (1922), but which normally occurs in ground water collections (e.g., shaded pools, channels, drains, pits and streams) in Ceylon, was found breeding in stumps of rubber, coconut, and kitul trees and giant bamboos on five occasions.



# ACKNOWLEDGMENT.

My thanks are due to Mr. H. F. Carter, Medical Entomologist, Ceylon, for affording me facilities for the work and for advice and assistance during its progress.

# REFERENCES.

- |                            |    |   |
|----------------------------|----|---|
| CARTER, H. F. (1925)       | .. | .. The anopheline mosquitoes of Ceylon. <i>Ceylon J. Sci. (D)</i> , <b>1</b> , pp. 19-59.           |
| GREEN, E. E. (1901)        | .. | .. Mosquitoes and malaria. Royal Botanic Gardens, Peradeniya, Circular Series 1, No. 25, p. 368.    |
| HACKER, H. P. (1922)       | .. | .. Annual Report, Malaria Bureau, F. M. S., for 1921.   |
| JAMES, S. P. (1914)        | .. | .. Report on a mosquito survey of Colombo. Government Sessional Paper No. XI. Govt. Press, Colombo. |
| SENIOR WHITE, R. A. (1920) | .. | .. A survey of the culicidæ of a rubber estate. <i>Ind. J. Med. Res.</i> , <b>8</b> , p. 304.       |



# MALARIA IN THE COASTAL BELT OF ORISSA.

BY

BRIGADIER G. COVELL, M.D., D.P.H., I.M.S.

(*Director, Malaria Institute of India*)

AND

ASSISTANT SURGEON (I. C.) PRITAM SINGH, I.M.D.

(*Late Officer-in-Charge, Orissa Malaria Inquiry, I. R. F. A.*)

[August 11, 1942.]

## CONTENTS.

	PAGE.
PREVIOUS INVESTIGATIONS .. .. .	458
PHYSICAL FEATURES .. .. .	458
Chilka Lake .. .. .	458
Coastal belt from Chilka Lake to Gopalpur .. .. .	463
CLIMATE .. .. .	464
RAINFALL .. .. .	464
GENERAL CHARACTERS OF VILLAGES AND THEIR INHABITANTS .. .. .	464
HISTORY OF MALARIA .. .. .	465
ROUTINE SPLEEN AND BLOOD EXAMINATIONS .. .. .	466
ANOPHELINE MOSQUITOES .. .. .	468
<i>A. sundaicus</i> .. .. .	469
Other anopheline species .. .. .	474
Dissections of anopheline mosquitoes .. .. .	476
SPECIAL INVESTIGATIONS .. .. .	477
Survey of villages on the eastern margin of Chilka Lake .. .. .	477
Survey of villages at the northern end of Chilka Lake .. .. .	479
Survey of villages south and south-west of Gopalpur .. .. .	479
Range of infiltration of <i>A. sundaicus</i> inland .. .. .	480
Range of infiltration of <i>A. sundaicus</i> south of Chilka Lake .. .. .	481
DISCUSSION .. .. .	482
CONTROL OF MALARIA IN THE ORISSA COASTAL BELT .. .. .	483
SUMMARY .. .. .	486
ACKNOWLEDGMENTS .. .. .	487
REFERENCES .. .. .	487
APPENDIX .. .. .	488



THE observations recorded in this paper were carried out during the period 22.iv.39 to 25.iii.42, at the request of the Government of Orissa, by a research unit from the Malaria Institute of India consisting of one Assistant Surgeon (the junior author), one laboratory assistant and 4 insect collectors. The part played by the senior author in the investigation was confined to the planning and general direction of the work and the analysis and presentation of the data collected.

The headquarters of the unit were located throughout at Rambha, on the Bengal-Nagpur Railway, near the southern end of the western shore of Chilka Lake. Detailed systematic observations were carried out throughout the period of the investigation from Sana Nairi, 24 miles north of Rambha, to Chatrapur, 16 miles to the south, whilst special expeditions to elucidate particular problems were made along the whole of the lake margin and as far south at a point on the coast opposite Vizianagram.

### PREVIOUS INVESTIGATIONS.

Results of observations carried out in the vicinity of Chilka Lake have been published by Fry (1912), Annandale and Kemp (1915), Sewell and Annandale (1922), Sarathy (1932), Senior White (1937) and Senior White and Adhikari (1939). The last named authors reviewed the previous literature in considerable detail. Fry's survey had revealed the significant fact that spleen rates were high in villages along the margin of the lake, and that they progressively decreased as the distance from it increased. This led him to suspect that *A. sundaicus*, the only malaria vector in India which habitually breeds in saline waters, might be the local transmitting agent. It was not, however, until 1936 that the presence of this species was established (Senior White, *loc. cit.*). This important discovery did much to clarify a situation which had previously been obscure, and afforded a probable explanation both for the periodical explosive epidemics which occur in this area, and for the local distribution of malaria. There were still a number of details to be investigated, however, to complete the picture, and it was with the object of filling in these gaps and formulating a control programme which might offer a reasonable prospect of success that the observations here recorded were undertaken. During the same period, researches dealing with the aquatic vegetation of the lake, which Senior White had shown to have an important bearing on the malaria problem, were carried out by Mr. P. K. Parija, Principal, Ravenshaw College, Cuttack, with the aid of a special grant from the Indian Research Fund Association.

### PHYSICAL FEATURES.

#### CHILKA LAKE.

The greater part of Chilka Lake lies in Puri District. Its south-western end extends into Ganjam District, which was formerly part of Madras Presidency, but has been included in Orissa Province since 1937. Properly speaking it is not a lake at all, but a lagoon, i.e., a body of salt or brackish water separated from the sea by low sand-banks. Its surface varies in extent from 350 to 450 square miles, depending on the fluctuations of the water level, the maximum length and breadth during the dry season being about 40 and 12 miles respectively. The sand-dunes



which separate it from the Bay of Bengal are in places only a few hundred yards in breadth. With the exception of Somolo, there are no inhabited islands along its western margin, but along the eastern border there are many such, separated from one another by narrow channels. This line of islands, together with a slender tongue of land which projects into its northern end, divides the main body of the lake from an outer channel about 12 miles long which connects it with the sea. The inner opening of this channel (locally known as *mugger-mukh*) is approximately opposite the lake's centre, and is extremely shallow. Its outer opening into the Bay of Bengal lies several miles further to the north, and is subject to considerable variations from year to year, both in size and position. In June 1940 it was not more than half a mile wide, and was intersected by three wedge-shaped bars of sand (Map 1).

During the monsoon months, the broad northern portion of the main body of the lake receives fresh water from the Daya and Bhargavi Rivers, whilst, during the dry season, its salinity becomes as great as that of the southern narrow portion, or even more so. In the monsoon, the water in the northern portion is rendered muddy by the river floods, and this probably exerts an unfavourable influence on the growth of aquatic vegetation by interfering with the penetration of light. The water at the southern end of the lake never becomes muddy, except near the mouths of nalas discharging into it, and its salinity, though somewhat reduced during the monsoon, remains comparatively high throughout the year.

The shore of the lake is very irregular, owing to the existence of numerous indentations or backwaters which favour the formation of swamps. Except at certain rocky points the margins are very shallow, the water being only a few inches deep, and sheets of putrefying floating weeds, *Potamogeton* and *Najas*, are formed on the surface. These are frequently bound together by algæ, mainly of the species *Lyngbya*. The shores of the narrow southern portion of the lake and of the islands are embanked in many places, to protect the fields from invasion by salt water.

During the dry season, the lake is said to be about 10 feet deep in its deepest part, near Kalijai Island. On 9.ii.40, the depth near Ghanta Silla Rock, the promontory opposite Sabilia, was 7 feet 6 inches. Throughout the period of our investigations, the water levels opposite Sabilia were recorded on a graduated pole, the zero mark on which was correlated with ordnance datum. The maximum levels recorded were :—

1939	..	.. 6 feet (9.ix.).
1940	..	.. 5 feet 8 inches (16.vii.).
1941	..	.. 5 feet (2nd and 3rd weeks of October).

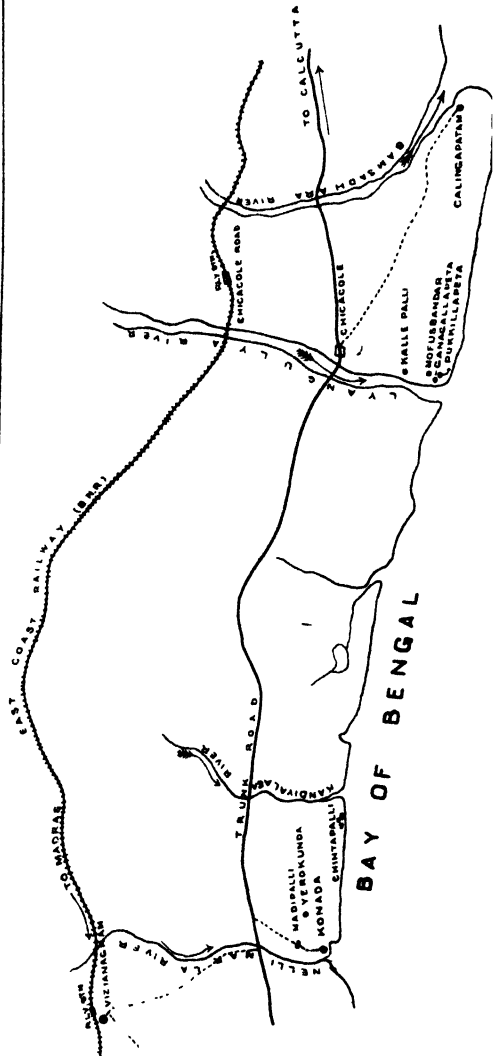
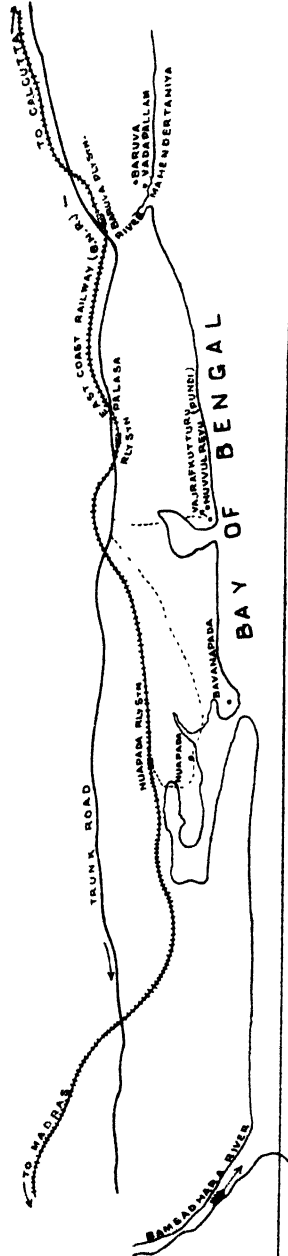
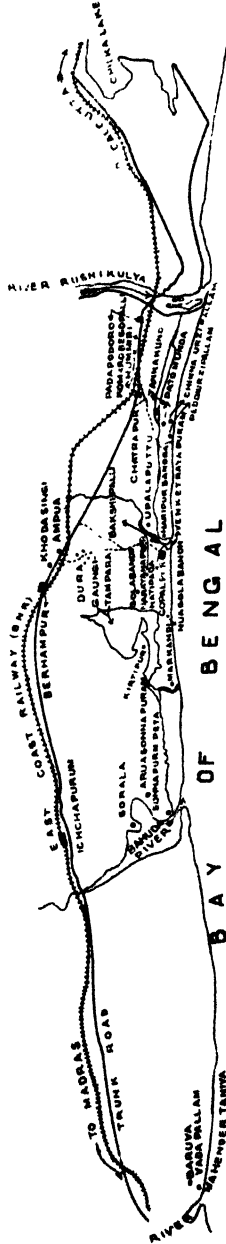
In 1940, as the result of the early onset of the monsoon and consequent river floods, the lake level began to rise earlier than in 1939, and the post-monsoon fall also commenced earlier, leaving behind it extensive swamps at Kesopur, Konoka and Proyagi. These afforded suitable breeding places for *A. sundaius*, and their presence accounts for the outbreaks of malaria which occurred in these villages in 1940, but not in 1939.







MAP 2.





A general idea of the variations in levels during the year was obtained from the figures recorded for 1940 and 1941. In the former year, the readings ranged from  $1\frac{1}{2}$  feet to nil during the period January to mid-May, only rarely falling below ordnance datum. From mid-May, there was a rise from 2 feet to a peak of  $5\frac{1}{2}$  feet in July, then a more or less stationary period till the end of August, after which there was a fall to about 2 feet, a level which was maintained with minor fluctuations throughout the last 3 months of the year. In 1941, the levels were much lower during March and April, when they were consistently at or below ordnance datum, the lowest reading being approximately 2 feet in the latter month. There was a rise during May to slightly above ordnance datum, and from June onwards the levels fluctuated between 3 and 5 feet until the end of the year. The above account indicates the general trend of the readings, but there were frequently abrupt changes of level from day to day, sometimes amounting to as much as 1 to 2 feet in 24 hours.

The degree of salinity in the lake is affected by the following factors\* :—

(1) *Floods in the Daya and Bhargavi Rivers*, the effect of which is to force the water from the northern portion of the main body of the lake partly into the outer channel and partly into the narrow portion south of Kalijai Island. The water in the northern portion is thus rendered almost completely fresh, whilst that in the southern portion becomes diluted and less saline.

(2) *Tidal influence*, which increases the lake's salinity during the dry season, whilst during the monsoon months it causes heading up of the flood waters. In the course of a survey of the islands along the eastern border of the lake in June 1940, it was observed that the tide encroaches as far as the margins of Tua and Garbai villages. During the driest months of the year (March, April and sometimes May), the tide also affects the salinity of the eastern bay at the southern end of the lake through the Chilka-Ganjam Canal.

(3) *Wind*. A moderately strong wind blowing from the north may raise the water level by as much as 2 feet opposite Sabilia, whilst a strong south-westerly wind exerts a reverse effect. This leads to an admixture of waters of different salinities.

(4) *Local rainfall* causes a certain amount of dilution.

(5) *Evaporation* tends to increase the salinity.

Records relating to salinity at different points were recorded in parts per 100,000 by the field method of Sinton and Kehar (1930), samples being taken at a distance of 4 or 5 yards from the shore (Table I and Graph).

Observations regarding the flow of water along the Chilka-Ganjam Canal indicate that, so long as the level of the lake remains about 2 feet above mean sea level, variations in the tide do not affect its salinity through this channel.

---

\* A detailed discussion of the factors affecting salinity in Chilka Lake was given by Senior White and Adhikari (*loc. cit.*).

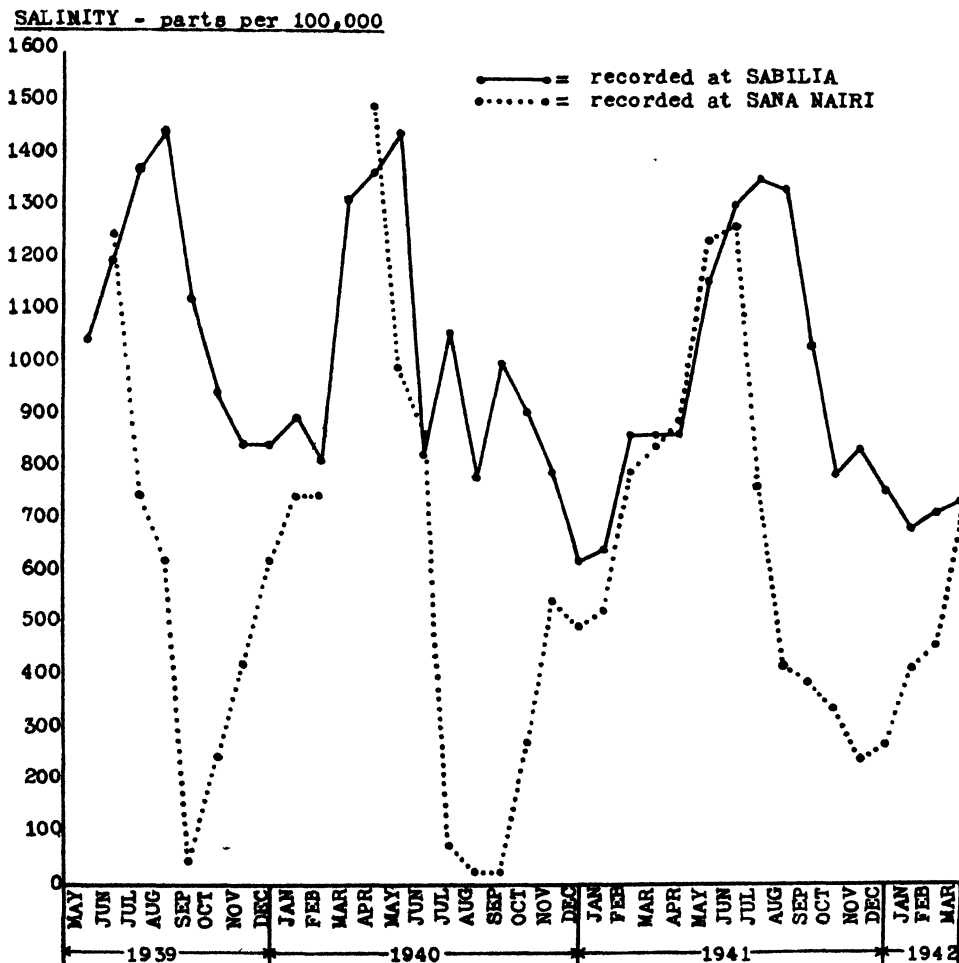


## COASTAL BELT FROM CHILKA LAKE TO GOPALPUR.

In former days, Chilka Lake was connected with Gopalpur Creek through the Chilka-Ganjam Canal, the Rushikulya River and the Old Navigation Canal. The Chilka-Ganjam Canal still exists, and is about 9 miles in length. It takes its origin

## GRAPH.

Records of salinity, Chilka Lake, May 1939 to March 1942.



from the eastern bay at the southern end of the lake near Lakhanapur, and terminates in the estuary of the Rushikulya River. The southern section of the Old Navigation Canal, which formerly connected the Rushikulya River with Gopalpur Creek, is still used for irrigation purposes. The northern portion expands into a



large body of water (*tampara*), 4 miles long by about 200 to 300 yards broad, which lies between Chatrapur town and the sea. At the tail end of this is a dam, through which excess water is passed by means of two sluice-gates and a waste-weir into a channel connected with the Rushikulya River. Hardly a trace of the central portion of the Old Navigation Canal now remains (Map 2).

Gopalpur Creek is about  $2\frac{1}{2}$  miles long, its maximum breadth being about 600 yards. It is affected by tidal influence from approximately July to March. In the latter month, the mouth of the creek becomes silted up by drifting sand, whilst during the rainy season the water from Kandla Nala and the Old Navigation Canal, supplemented by local rain, accumulates until Haripur and Bandra villages, to the north of the creek, are threatened with flooding. The local inhabitants then make a breach in the sand-bar, and the water is drained away. This opening is not made earlier, because (i) the sand-bar affords a means of access to Gopalpur for the inhabitants of Haripur and Bandra, and (ii) if the breach is made too early there is a risk that it may again be closed by sand-drifts, in which case the work would have to be repeated.

#### CLIMATE.

The weekly averages of maximum and minimum temperatures and relative atmospheric humidity from July 1939 to March 1942 recorded at Rambha are given in Table II. The maximum temperature rarely exceeds  $100^{\circ}\text{F}$ . April, May and June were the hottest months, the highest temperature recorded during the 3 years being  $106^{\circ}\text{F}$ . on 20.vi.41. The lowest figure recorded was  $51^{\circ}\text{F}$ . on 25.xii.40 and 10.i.41.

The average weekly 4 p.m. relative humidity figures, which are of more importance as an indication of conditions favouring malaria transmission than those recorded at 8 a.m., never reached 70 per cent during the year 1940, although this was a year of exceptionally heavy rainfall. During 1941, however, this figure was in the neighbourhood of 70 per cent from the second week in June to the end of October.

#### RAINFALL.

The figures from 1929 onwards are given in Table III. From July 1939, these were recorded at the headquarters of the research unit at Rambha, whilst for the decade prior to this they have been abstracted from the records maintained at Rambha police station. The average annual rainfall during the 13 years was approximately 43 inches, the maximum precipitation being 60.37 in 1940 and the minimum 24.54 in 1934. The greater part of the rain falls during the period May to October. The effects of early and heavy rainfall and consequent floods in the Daya and Bhargavi Rivers on the level of the lake and the formation of swamps along its margins, such as occurred in 1940, have already been noted.

#### GENERAL CHARACTERS OF VILLAGES AND THEIR INHABITANTS.

Generally speaking, housing conditions in the villages are poor. Most of the houses have mud walls and thatched roofs, though there are a few belonging



to well-to-do residents which are built of brick with tiled roofs. Food and housing conditions are of a higher standard at Chatrapur than elsewhere in the neighbourhood.

The population consists mainly of Oriyas, whose chief occupation is agricultural, though there are a number of fishermen among them. Most of the railway personnel and some of the clerks at Chatrapur are Telegus, and a certain number of Telegus have settled in Ganjam District. There is an Anglo-Indian colony at Gopalpur, which has a reputation as a seaside health resort. The economic condition of the population as a whole is poor, especially in years when cyclones cause the saline water of the lake to overtop the embankments and spoil the rice, only one crop of which is harvested during the year.

The importance of Chatrapur lies in the fact that it is the headquarters of the district, and a reserve police force is stationed there. At Humma, there is a salt factory, the employees of which are mostly Telegus. Gopalpur is a port, as well as a health resort.

### HISTORY OF MALARIA.

The villages in the vicinity of Chilka Lake and its connecting canals have been subject to periodical epidemics of malaria from as far back as the available records extend. Ganjam, the former headquarters of the district, is said to have been devastated by a virulent outbreak of fever in 1815. Chatrapur, the present headquarters, was, according to local reports, a good deal healthier half a century ago than at the present time. It is stated that the incidence of malaria has progressively increased during the last two decades, and this has been ascribed to the introduction of canal irrigation and also to the development of casuarina plantations in the neighbourhood. There was formerly a belt of thick jungle between the tampara and the town, which may have acted as a barrier against the infiltration of mosquitoes, but we were unable to ascertain the date when this was cleared away.

Reports of previous investigations and the observations here recorded indicate that the tract is subject to periodic malaria epidemics which are very patchy in their distribution, so that one village may suffer severely, whilst in another nearby there may be no abnormal rise in malarial incidence.

As stated above, Fry (*loc. cit.*) reported the existence of high spleen rates in the immediate vicinity of Chilka Lake, and noted that these became progressively lower as the distance from the lake increased. In 1928 and 1930, the Special Malaria Officer, Madras, surveyed the Humma group of villages. Observations were also carried out in the vicinity of the lake by Sarathy in 1931-32. None of these observers recorded the presence of *A. sundaicus*, although they were all on the lookout for this species. It seems probable that their investigations were carried out in each case either in a non-epidemic year, or at a time when an epidemic was subsiding and the vector species was no longer present. A striking example of the abrupt manner in which *A. sundaicus* may disappear at the close of its season of prevalence was observed at Sabilia in the first week of May 1940, and this accords with the experience of Covell (1927a) in the Andamans. There is usually an unavoidable delay before an investigation can be commenced in any



area where an epidemic of malaria has been reported. It is not, therefore, very surprising that the presence of this species in the Orissa coastal belt was not actually proved until specimens were collected by Senior White at Konoka village, which lies near the lake margin between Rambha and Kallikota railway stations, on 31.iii.37, and at Kallikota station on the following day.

The dispensary statistics relating to malaria cases treated at Kallikota, Rambha, Ganjam, Chatrapur and Gopalpur over a number of years are given in Tables IV to VIII. These show that outbreaks of malaria may occur either in the spring or autumn months. At Kallikota, there was a fairly severe spring rise in 1931, after which the incidence of malaria was low from 1932 to 1936 inclusive, but there were severe epidemics in the spring months of 1938, 1939 and 1941. There were fairly severe outbreaks in the autumn months in 1937 and 1940, but these were of much less intensity than the spring rises above noted.

At Rambha, there were outbreaks of some intensity in the spring months of 1936 and 1937 and to a lesser extent in 1940 and 1941. The autumn rises in 1936, 1937 and 1940 were also of marked severity.

At Ganjam, there was a very severe epidemic in the spring of 1937, with a peak in April and a second in July. In 1938 malaria was prevalent during the same period, with peaks in April and September. In the following year, there was no spring rise, but in the autumn there was another severe outbreak with a peak in November. In 1940, the most malarious months were June and December and the epidemic continued until June of 1941 with a peak in May. The autumn rise in that year was very slight.

Chatrapur experienced fairly severe outbreaks in the spring and autumn of 1931. After this the incidence of the disease was slight until 1937 and 1938, in each of which years the most malarious month was July. In 1939 there was a minor peak in March, but no autumn outbreak, whilst in the following year there was no rise in the spring but a large increase in cases in November and December.

At Gopalpur, there is evidence of a spring rise in certain years, but there appears to be usually a greater incidence in the autumn, particularly noticeable in the years 1931 and 1937.

#### ROUTINE SPLEEN AND BLOOD EXAMINATIONS.

The results of these investigations are shown in Table IX, and relate to 33 different localities extending from Soran to the north on the western shore of Chilka Lake to Gopalpur Creek to the south. Spleen examinations were carried out on as many children as could be collected, and blood examinations on 50 children in most instances, the same individuals being examined on each occasion as far as possible. The highest figures were recorded at Kesopur in the winter of 1940-41, when the spleen rate was in the neighbourhood of 100 per cent for several months. The parasite rate in November 1941 was 72 per cent, with a gametocyte rate of 31 per cent and an average parasite count of over 2,000 per c.mm. of blood. In the early part of the malaria seasons and at the height of an epidemic, the infections



were almost all *P. falciparum*, whilst, as the epidemic was subsiding, a certain proportion of *P. vivax* and *P. malariae* were encountered. Infections with *P. malariae* were particularly numerous at Dubrakudi and Haripur-Bandra during the period May to September.

As regards spleen rates, the villages in the Chilka Lake area fall into two categories: (i) those in which the rates do not show any great reduction in non-epidemic years (e.g., Proyagi, Dubrakudi, Konoka and Kesopur), and (ii) those in which the rate falls very markedly in the inter-epidemic period (e.g., Soran, Sana Nairi, Balugaon and Sabilia). In the former case, the enlarged spleens are the result of repeated attacks of malaria, and they have become so large and so fibrosed that the absence of an outbreak for one or two years causes no appreciable diminution in the spleen rate.

The figures indicate that during March and April 1939 there were epidemics of malaria at Soran, Sana Nairi, Sabilia and possibly Dubrakudi, but the research unit did not arrive in Orissa until these had subsided. During the remainder of the year 1939, there was no evidence of malaria transmission in the localities under study.

During 1940, there were two transmission seasons, the first in March-April and the second in the autumn months, commencing towards the end of September. In the March-April epidemic, Sabilia was the village chiefly affected, but mild outbreaks occurred in Konoka, Dubrakudi and Humma. The autumnal epidemic affected Kesopur, Konoka, Sabilia, the Dubrakudi group (including Lakhanapur and Proyagi) and Humma. In the remaining villages under observation there was no outbreak of malaria between May 1939 and December 1940.

In 1941, there was a spring outbreak in most of the villages under observation, and this was followed in the autumn of the same year by a more severe and widespread epidemic, affecting particularly the villages north of Balugaon, as far as Bhusandpur. At Balugaon itself, there was no noticeable outbreak of malaria between the spring of 1939 and that of 1942. At Chatrapur, there was a slight rise in malaria incidence in March 1941, and a more severe outbreak from July to September. This was chiefly limited to the inhabitants of certain huts near the tampara and to the part of the town nearest to it. At Gopalpur and Upplaputtu, there was a slight rise in malaria incidence from July-August onwards. In the Ganjam area, Boropalli was affected in the spring and Pallibondho and Purana-bondho in the autumn whilst Ganjam itself escaped in each case.

The sudden apparent rise in spleen rate recorded at Garh Humma in May 1940 was largely due to the inclusion of children of low caste from a group of huts near the village. The spleen rates of the two communities shown separately were:—

	Number examined.	Spleen rate, per cent.	Average enlarged spleen.
Garh Humma ..	30	60	7.8
Adjacent huts ..	10	90	6.8



The consistently high spleen rates recorded in this village as compared with Tolo Humma may be due to the proximity of the former to the railway station, to which specimens of *A. sundaicus* are liable to be carried by trains from Kallikota station, which serves Kesopur village. It is noteworthy that during the period October 1940 to January 1941, when the density of *A. sundaicus* at Kesopur was very high, Garh Humma suffered severely from malaria, the spleen rate rising from 48 to 80 per cent. From February 1941 to March 1942 the density of *A. sundaicus* at Kesopur was low, no specimens were captured at Garh Humma and the spleen rate in the latter village had declined to 53 per cent at the end of this period. At Kotarakuda, a village on the sea coast opposite Humma, the spleen rate in May 1941 was 67 per cent.

There was a rise in spleen rate at Hummuri village from 11 per cent in May to 41 per cent in July 1940. No observations were made in this village in June of that year, when the unit was engaged on a special investigation elsewhere, and the existence of the outbreak was not discovered until it was visited on July 17. On this and subsequent occasions intensive search was made for *A. sundaicus* without success.

Certain special investigations were made in the Chatrapur area in November 1940, following the discovery of a few specimens of *A. sundaicus* in the town itself. At Pedda Urzipallam, a village on the sea shore 4 miles from Chatrapur, the spleen rate was 28 per cent (100 examinations). The only local water collections were wells and some casuarina pits, and it is probable that the incidence of malaria is chiefly due to the fact that the inhabitants visit the tampara at night to fish. The only anopheline larvæ found in the vicinity of the village were those of *A. hyrcanus* and *A. subpictus*. Five children living in two huts at Patomanda near the origin of the tampara, 2 miles south-east of Chatrapur, were all found to have enlarged spleens. At Tankakun, a group of huts on the margin of the tampara nearest the sea and one mile south-east of Chatrapur, the spleen rate was 75 per cent. Adult specimens of *A. sundaicus* were caught in both groups of huts, though only one of this species hatched out from more than 4,000 larvæ collected in the tampara itself during the month.

#### ANOPHELINE MOSQUITOES.

The following 17 species have been encountered during the investigation :—

<i>A. aconitus.</i>	<i>A. pallidus.</i>
<i>A. annularis.</i>	<i>A. ramsayi.</i>
<i>A. barbrostris.</i>	<i>A. stephensi.</i>
<i>A. culicifacies.</i>	<i>A. subpictus.</i>
<i>A. fluviatilis.</i>	<i>A. sundaicus.</i>
<i>A. hyrcanus.</i>	<i>A. tessellatus.</i>
<i>A. jamesi.</i>	<i>A. vagus.</i>
<i>A. karwari.</i>	<i>A. varuna.</i>
<i>A. maculatus.</i>	



In the villages under intensive study, three classes of catching station were established: (A) human dwellings; (B) huts occupied by both human beings and cattle; (C) cattle-sheds. The total number of each species collected monthly in the various classes of catching station in each village is given in Tables X-a to X-k. Collections were made thrice weekly during 1939, approximately the same time being devoted to each day's search, but in 1940 and 1941, when investigations were extended further afield, it was not always possible to make collections as frequently as this. In order to make possible a more accurate comparison of the densities of the various species in the different villages throughout the period, the average daily catch per week of each species was calculated, by dividing the total weekly catch by the number of collections made during the week. It is impossible to tabulate figures for all species, but the figures relating to *A. sundaicus* are given in Table XI.

Larval collections were made twice weekly in each village from all breeding places within half a mile from the periphery.

#### *A. SUNDAICUS.*

##### *Adult collections.*

A study of the catches made in the different villages shows that the presence and density of *A. sundaicus* in this area are extremely variable. It may be prevalent in any particular locality either in the spring or autumn, or at both these times. It may then disappear, possibly for years, whilst, on the other hand, it may suddenly make its appearance in a place where its presence had never before been recorded.

At Sana Nairi, where intensive observations were carried out from July 1939 onwards, *A. sundaicus* was not encountered until January 1940, when a single specimen was caught. From then until the end of September 1940, the total captured was 11. From October onwards, there was a progressive increase in density, and in the second week of February 1941 the average daily catch exceeded 90. During the latter half of April 1941, the numbers fell rapidly and from May to September the species practically disappeared from the locality. It reappeared in large numbers in October and continued to be abundant until the investigation terminated in March 1942.

The figures relating to Balugaon present a striking contrast with those from Sana Nairi, for during the whole period May 1939 to March 1942 the total number of *A. sundaicus* captured was only 23, and 10 of these were caught in a single month (March 1941). These figures are of interest because there had been a violent outbreak of malaria in this village in August 1936, recorded by Senior White (*loc. cit.*). No collections were made at Balugaon until the epidemic had subsided, and no specimens of *A. sundaicus* were found there during the months immediately following the epidemic, but there can be little doubt in the light of other evidence that this species was the vector concerned.

At Kesopur, *A. sundaicus* was almost completely absent from June 1939 to the second week of August 1940. There was then a sudden and rapid rise in density,



the total number collected in October exceeding 2,000, with an average daily catch of 138 in the third week. There was an abrupt drop in numbers in the latter half of February, and from March to September the species was almost completely absent. It reappeared in the autumn of 1941, but in very low density compared with the previous year.

At Konoka, 4 specimens were captured in May 1939 and one in June. During March and April 1940, 38 specimens were caught, but apart from this the species was almost entirely absent till the autumn of that year when the density increased, the average daily catch in the third week of November being 30. After this the numbers fell nearly to zero, and in the following autumn there was no appreciable rise.

At Sabilia, *A. sundaicus* was present in considerable numbers in April and May 1939. It then abruptly disappeared till the spring of 1940, when it was again fairly abundant, the average daily catch in the second week of April exceeding 60. After this the species practically disappeared until October, when it was again present, though in fewer numbers, up to March 1941. During the succeeding 12 months, it was almost completely absent.

At Tolo Humma, a single specimen of *A. sundaicus* was caught in May 1939, but none during the remainder of the year. From January 1940 to March 1941 a few specimens were caught each month, with an appreciable rise during the period October to January, the total catch for these 4 months being 135. From April 1941 to March 1942 the species was almost entirely absent.

At Dubrakudi, *A. sundaicus* was not encountered from May 1939 to January 1940. A few specimens were caught each month from February to October 1940, and there was a sudden increase in density towards the end of the latter month, with an average daily catch of 51 for the first week of November. There was then an abrupt fall in density, and the species did not reappear in this locality in the autumn of 1941.

At Chatrapur, only 7 specimens of *A. sundaicus* were collected from May 1939 to October 1940. In November 1940, 18 were caught, but the December catches were all negative. A few specimens appeared in the collections made during the next 6 months, and in July and August 1941 there was an appreciable rise in density, with an average daily catch during the first two weeks of August of 17 and 10, respectively. During the remaining 7 months of the investigation, the catches were almost entirely negative. Almost all the specimens of *A. sundaicus* captured in this locality were from huts in the immediate neighbourhood of the tampara.

#### *Daytime resting places.*

Out of 16,938 adult specimens of *A. sundaicus* captured during the investigation, 8,835 were caught in huts occupied only by human beings, 4,237 in huts occupied both by human beings and cattle, and 3,866 in cattle-sheds. Whilst this gives only an approximate idea of the preferential daytime resting place of this species, the fact that over 77 per cent of the adult catch was made in rooms in which human beings spent the night, is suggestive.



*Larval collections.*

Since it is not possible to distinguish the larva of *A. sundaicus* with certainty, the identification was in all cases based on the examination of adult specimens hatched out from them. The chief breeding places of the species together with the salinity figures recorded and the type of aquatic vegetation present in them are shown in Table XII.

*Breeding places.*

The principal breeding places of *A. sundaicus* in this area are: (i) Chilka Lake itself, and (ii) tanks, pools, swamps and ricefields which are subject to flooding by saline water derived either from the lake or from some creek or back-water connected with the sea. In some inland villages, it has been found breeding in water collections of low salinity which are never flooded with sea water.

North of Kalijai Island, larvæ of *A. sundaicus* were present in the lake in large numbers from January to April 1941 opposite Sana Nairi, and in March and April of that year small numbers were collected off Balugaon. From October 1941 to March 1942, extensive breeding of *A. sundaicus* was observed along the whole shore line from Lambodarapur to Soran. At this time there was an exceptionally heavy growth of weeds in this section of the lake.

South of Kalijai Island, the number of *A. sundaicus* hatched out from larvæ captured in the lake was extremely small (Table XIII). All the larvæ of *A. sundaicus* taken in this part of the lake were collected near the shore in embayments in its margin. The growth of weeds in this area was never very extensive during the period of the investigation, and was almost entirely limited to indentations in the shore.

The principal factor affecting the breeding of *A. sundaicus* is undoubtedly the presence or absence of putrefying masses of algæ or other weeds. Salinity probably operates only in so far as it affects the growth of the weeds. Another factor which has an adverse effect on the breeding of this species in large bodies of water is wave action, which operates partly by disturbing the water surface, and partly by stranding vegetation on the shore.

*Association with aquatic vegetation.*

The association of breeding of *A. sundaicus* with putrefying masses of algæ and other weeds has been very striking throughout the investigation, and it has been a rare experience to find larvæ of this species in breeding places devoid of such masses. Vegetation actually growing from the bed of water collections has no apparent association with the breeding of *A. sundaicus*; it is only when such vegetation comes above the water surface and begins to putrefy that the larvæ of this species are found. The breeding of *A. sundaicus* was not observed to be associated with any particular type of algæ or other weeds. The species will apparently breed in the presence of many different types of aquatic vegetation,



provided that putrefaction is taking place. A typical instance of the course of events observed is given below :—

At Sana Nairi, the breeding of *A. sundaicus* was first observed in September 1940 in a small tank containing masses of *Cladophora* and *Cedogonium*. Larvæ of this species were collected in small numbers until November, but disappeared in December, when there was a marked change in the aquatic vegetation, only traces of the algæ remaining, although there was a considerable growth of *Lemna*. In the same month larvæ were collected from the lake itself, which now contained algal masses consisting of *Charatomorpha*, *Anabæna* and *Spirogyra*, together with a sheet of *Potamogeton* which extended about 100 yards out into the lake. During February 1941, there was a great increase in the putrefaction of the algal masses, and this was accompanied by a corresponding increase in the breeding of *A. sundaicus*, which was now very extensive. In March, a large proportion of the weeds was removed by the cultivators for use as manure. Breeding of *A. sundaicus* was greatly diminished, the reduction in numbers of larvæ being aided by wave action due to strong winds. In April, scanty breeding continued in the lake and a few larvæ were found in a disused boat on the shore and in a pool in the bed of a nala nearby. Larvæ were also found in a large tank containing *Ceratophyllum*, *Hydrilla*, *Nymphæa*, *Lyngbya* and *Oscillatoria*. Breeding of *A. sundaicus* ceased completely in the lake by the end of April, but continued in the tank during the early part of May, after which it ceased there also.

No larvæ of this species were subsequently found in this locality till October 1941, when a heavy growth of weeds, mainly *Potamogeton*, appeared all along the shore from Lambodarpur to Soran. This was so thick that the water surface was not visible for miles, and *A. sundaicus* was breeding in large numbers along the whole shore. No such growth of weeds had been hitherto observed during the whole of the investigation either here or elsewhere. In February and March 1942, when the putrefaction of the weeds increased, the numbers of larvæ of *A. sundaicus* increased also. In January of this year, larvæ of this species were also collected from a local tank in which they had never before been found.

#### *Range of salinity of breeding places.*

The optimum range of salinity appears to be from 600 to 800 parts per 100,000. In 1940, the highest figure recorded was 1,300 parts per 100,000, in the lake opposite Proyagi, whilst the lowest was 40 parts per 100,000 in a tank in Sana Nairi village in September. In October, the salinity in this tank rose to 230 and in November to 300 parts per 100,000 and the numbers of *A. sundaicus* larvæ showed a marked increase. It appears that an increase of salinity above the optimum range has a greater inhibitory action than a decrease of salinity below it, the effect of a salinity above the optimum being abrupt, whereas that of a salinity below the optimum is not so well defined. In a pool near Konoka in September 1940, in two tanks in the Sabilia-Rambha area in May 1940, and in a tank in Proyagi in March and April 1940, the salinity recorded exceeded 1,000 parts per 100,000, and the percentage of *A. sundaicus* larvæ collected was very low; whereas in a ricefield at Kesopur in August 1940, in two tanks in Sabilia in



May 1939, in a well at Humma in October 1940, and in a tank and a pool in Dubrakudi in March and April 1940, a much greater density of this species was found associated with salinities of 40 to 300 parts per 100,000. Tank 9 at Dubrakudi forms an exception to this rule, but the putrefying algæ here were scanty in amount.

In 1941, the minimum salinity in which larvæ of *A. sundaicus* were found, was 15 parts per 100,000 and the maximum 1,300 parts.

*Association with culicine breeding.*

Wherever the breeding of *A. sundaicus* is intensive, culicine larvæ have invariably been present, as is to be expected in water polluted by the presence of the putrefying masses of algæ and weeds.

*Association with malaria outbreaks.*

When the figures relating to adult mosquito collections are analysed in relation to the outbreaks of malaria which occurred in the villages under observation during the 3 years' investigation, the correlation between the prevalence of *A. sundaicus* (Table XI) and malarial incidence (Table IX) is very marked. There was no instance in which an epidemic occurred in the absence of *A. sundaicus*, provided that collections were made before the subsidence of the outbreak. Conversely, there was no instance of *A. sundaicus* appearing in a village in appreciable numbers without a corresponding outbreak of malaria following in its wake. The sudden disappearance of this species towards the end of an epidemic was observed repeatedly, and it is this phenomenon which accounts for the fact that *A. sundaicus* was not recorded from this area prior to 1937.

As regards the villages under routine observation:—

Soran experienced an epidemic in the spring of 1939. There was no further outbreak till the autumn of 1941, when the parasite rate rose from 6 per cent in October to 79 per cent in December. The density of *A. sundaicus*, which has been almost entirely absent during the whole period, rose from an average of 1 per collection in the last week of October to 66 in the first week of December.

Sana Nairi experienced an epidemic shortly before the commencement of the investigation. There was no further outbreak till April 1941, when the spleen rate rose from 48 to 70 in the space of a month, and continued to rise until the termination of the investigation in March 1942. The epidemic was preceded by a sudden rise in density of *A. sundaicus* to an average of 90 per collection in the second week of February, and this species, which had been either entirely absent or present only in scanty numbers prior to this date, remained numerous until the unit was withdrawn.

The case of Balugaon has already been discussed. *A. sundaicus* was almost completely absent from this village throughout the 3 years' investigation, and during this time there was no outbreak of malaria.



Kesopur, a hyperendemic village, experienced an epidemic in the autumn of 1940, when the parasite rate rose from 6 per cent in September to 72 per cent in November. *A. sundaicus*, which had been almost completely absent till the end of July 1940, appeared during August and reached an average of 138 per collection in the third week of October. From March to October 1941, the species practically disappeared, but there was a moderate rise in density in the autumn to about 30 per collection. This was followed by a rise in the parasite rate from 14 to 36 per cent.

Konoka experienced a mild outbreak of malaria in the spring of 1940, accompanied by a slight rise in the density of *A. sundaicus* to 3.7 per collection in the second week of April. A rather more severe outbreak in the autumn was accompanied by an increase in density of *A. sundaicus* to 30 per collection in the fourth week of November.

A malaria epidemic was in progress in Sabilia and Rambha when the investigation began in April 1939, at which time *A. sundaicus* was present in considerable numbers (57 per adult collection). The species disappeared with the subsidence of the epidemic, and was almost entirely absent until the second week of April 1940, when the density rose abruptly to 60 per collection, falling again to practically nil by the second week in May. The parasite rate rose from 2 per cent in March to 20 per cent in May, and the spleen rate from 33 per cent in March to 60 per cent in June. During the following 12 months, the adult catches of *A. sundaicus* remained low, and the amount of malaria transmission was correspondingly moderate.

At Tolo Humma, catches of *A. sundaicus* were consistently low throughout the period of the investigation, and only mild outbreaks of malaria occurred in this locality.

At Dubrakudi, catches of *A. sundaicus* were low except in the first week of November 1940, when the average per collection suddenly rose to 51, falling to 15 in the following week. The parasite rate rose from 12 per cent in October to 34 per cent in January. This is a hyperendemic village, with a spleen rate which never fell below 62 per cent during the 3 years' investigation.

At Chatrapur, there were a few cases of malaria in the spring of 1941, and a slightly more severe outbreak occurred in the autumn of that year, chiefly confined to the occupants of certain huts near the tampara. These outbreaks were accompanied by a localized increase in the density of *A. sundaicus* in the huts where the cases occurred. The species had been almost entirely absent from this locality during the first 2 years of the investigation.

#### ~ OTHER ANOPHELINE SPECIES.

*A. aconitus*.—This species was met with in all the villages under study, usually in very scanty numbers. Adult collections were higher at Chatrapur than elsewhere, but even here it formed only a minute proportion of the total catch. The great majority of specimens were caught in cattle-sheds, or in huts occupied both by human beings and cattle. Adult specimens were, however, regularly collected from



the Tankakun group of huts near Chatrapur tampara, where there were no cattle-sheds. Its period of greatest prevalence was in November and December. The chief breeding place was in pools among fallen paddy. At Chatrapur, the larvæ were also found in certain parts of the tampara, where the vertical vegetation (*Scirpus*) had fallen. In the case of paddy, this stage (of falling) is reached when the water in the fields is beginning to dry up and the crop is ripening. *A. aconitus* has also been found breeding in wells, borrowpits, tanks and pools.

*A. annularis*.—Adults were present in considerable numbers in all villages, the period of greatest prevalence being from July to December. The great majority of specimens were caught in buildings occupied by cattle. This species breeds profusely in tanks, borrowpits and swamps, in association with *Hydrilla*, *Najas* and *Ceratophyllum*. Its most prolific breeding places were the tampara at Chatrapur and the Jampana tank at Sana Nairi. On rare occasions larvæ have been collected from the lake itself, and from wells.

*A. barbirostris*.—This species is rare throughout the area under study. The great majority of adult specimens were collected in cattle-sheds. It breeds in shaded water collections, tanks containing *Pistia* and other rank vegetation, pools in casuarina plantations, paddyfields and wells.

*A. culicifacies*.—This species was present in all villages, but nowhere in large numbers. A large proportion of the adult specimens captured were either from cattle-sheds or from huts occupied both by human beings and cattle. Its period of greatest prevalence was from July to September. Its favourite breeding places were rainwater collections, nalas, borrowpits, drains in engine yards, irrigation channels at Chatrapur and wells. On rare occasions the larvæ were collected from tanks, paddyfields and quarry-pits.

*A. fluviatilis*.—One adult specimen was caught at Sabilia, and one at Balugaon. No larvæ were encountered.

*A. hyrcanus*.—This species is almost as rare in this locality as is *A. barbirostris*. Most of the adult specimens collected were from cattle-sheds. Generally speaking, its breeding places were the same as those of *A. barbirostris*. Its larvæ were also found occasionally in the tampara at Chatrapur.

*A. jamesi*.—Adult specimens were captured at Kallikota (1), Ganjam area (1), Sana Nairi (2), Kesopur (3) and Chatrapur (2). A few specimens were bred out from larvæ collected from a tank and a borrowpit at Humma, from tanks at Ganjam, and one specimen from the tampara at Chatrapur.

*A. karwari*.—Larvæ were found in seepage water near the playing field at Chatrapur. No adult specimens were collected.

*A. maculatus*.—Two adults were collected, one from Soran, the other from Balugaon. No larvæ were encountered.

*A. pallidus*.—Specimens were collected in all villages, but nowhere in large numbers. Its period of greatest prevalence was November. The adults were mostly caught in cattle-sheds. Larvæ were found chiefly in ricefields. This species was also found breeding in tanks and borrowpits in association with *A. annularis*.



*A. ramsayi*.—This species was encountered in most villages, but in very scanty numbers. The majority were caught in cattle-sheds. The larvæ were found in tanks and pools containing *Pistia*, and, rarely, in borrowpits.

*A. stephensi*.—One adult specimen was caught in a cattle-shed at Chatrapur. No larvæ were encountered.

*A. subpictus*.—This was the commonest species in all villages. The majority of the specimens collected were from buildings occupied by cattle, but large numbers were found in human dwellings also. Its period of greatest prevalence was from June to November. Larvæ were found in almost every type of breeding place.

*A. tessellatus*.—A few specimens were captured in most of the villages, on rare occasions, but no larvæ were collected.

*A. vagus*.—This species was collected in all villages in considerable numbers, its period of chief prevalence being September and October. Its daytime resting places and breeding places were the same as those of *A. subpictus*.

*A. varuna*.—This species was found in most villages, but in scanty numbers only. The majority of specimens were caught in cattle-sheds or huts occupied by both human beings and cattle. Its period of chief prevalence was from November and December. The larvæ were found in wells, borrowpits, pools, tanks and in the terminal portion of the irrigation canal at Chatrapur. One specimen was collected from the tampara in this locality.

#### DISSECTIONS OF ANOPHELINE MOSQUITOES.

The total number of each species dissected and the numbers found infected are given in Table XIV. With the exception of a solitary gut infection in a specimen of *A. annularis* collected from Sana Nairi in August 1940, the only species found infected during the 3 years' period of investigation was *A. sundanicus*. All the infected specimens of this species were collected in villages where active transmission of malaria, as evidenced by dispensary figures and blood examinations, was occurring, except for one gut infection in a specimen caught at Chatrapur in July 1940. Of the total number of *A. sundanicus* dissected, 6,085 were caught in human dwellings, 2,525 in huts occupied both by human beings and cattle and 2,104 from cattle-sheds. All the infected specimens were caught from the first two types of resting places, except one with a gut infection which was collected from a cattle-shed at Dubrakudi.

The months during which infected specimens of *A. sundanicus* were found and the localities in which they were captured are given in Table XV. Forty-eight of the 82 infected specimens were caught during the months of September to December, and 30 during the months of January to May. The months in which the greatest number of infected specimens was captured were October (21) and February (14). The infection rates were low throughout, except at Kesopur in February 1941, when the percentage infected was 9.2 (65 dissections, gut infected 5, gland infected 1). With this exception, the maximum monthly infectivity rate in *A. sundanicus* during the height of the transmission period in villages where epidemics were in progress, was usually about 1.5 to 3.0 per cent.



## SPECIAL INVESTIGATIONS.

## SURVEY OF VILLAGES ON THE EASTERN MARGIN OF CHILKA LAKE.

A rapid malaria survey in this area was carried out in June 1940. The villages visited are situated either on the narrow tongue which projects from the mainland at the north-eastern corner of the lake, or on the chain of islands which lie along its eastern border. Fry (*loc. cit.*) had reported that these villages were intensely malarious, and from his remarks as to the high degree of tolerance which the inhabitants had developed and their general attitude towards the disease, which they apparently regarded very lightly, it was surmised that the high spleen rates recorded by him represented a condition of true hyperendemicity rather than the aftermath of a recent epidemic. The object of the expedition was to confirm Fry's observation, and if possible to determine the identity of the vector species of anopheline.

The results of spleen examinations made in the villages visited, together with the figures recorded by Fry in those in which he made observations, are given in Table XVI.

Notes on individual villages are given below :—

Garh Krishnaprasad is on Parikud Island, which lies opposite a promontory on the western margin of the lake midway between Balugaon and Kesopur. There are a number of ricefields between the lake and the village. The inhabitants stated that during a cyclone in October 1938 the lake water encroached up to the periphery of the village, rendering the water in certain tanks brackish. Two larvæ of *A. sundaicus* were collected from a tank with salinity 25 parts per 100,000. Larvæ of *A. subpictus* and *A. annularis* were also collected. Adult mosquito collection : *A. sundaicus* 19, *A. subpictus* 30, *A. culicifacies* 1, *A. hyrcanus* 1. Spleen rate 94 per cent.

Khatiakudi is on the lake shore, half a mile from Garh Krishnaprasad. When the level of the lake falls each year numerous pools and swamps are left close to the village. The only larvæ collected were *A. subpictus*. Adult mosquito collection : *A. sundaicus* 2, *A. subpictus* 45, *A. annularis* 1. Spleen rate 100 per cent.

Garbai is on another island north of Parikud, and lies about 10 miles distant from Garh Krishnaprasad, about 300 yards from the shore. Salt was formerly manufactured there and the industry is now being restarted, after a lapse of about 40 years. Larvæ of *A. subpictus* and *A. pallidus* were collected. Adult mosquito collection : *A. subpictus* 45, *A. vagus* 1. Spleen rate 96 per cent.

Mohasa is on an island immediately south-east of Garbai. It is a small village of about 40 houses, inhabited chiefly by fishermen. There are collections of brackish water in two nalas near the village, and in a tank on the lake shore. One larva of *A. sundaicus* was collected from a tank with salinity 45 parts per 100,000. Larvæ of *A. subpictus* and *A. vagus* were also collected. Adult mosquito collection : *A. subpictus* 68. Spleen rate 87 per cent.

Poradihi is about 7 miles north of Garbai, on a spit of land projecting from the mainland. Salt was manufactured here till about 40 years ago. There was



an epidemic of malaria here in April 1940. The only larvæ collected were those of *A. subpictus*. Adult collection: *A. sundaicus* 2, *A. subpictus* 33. Spleen rate 88 per cent.

Tua is another small village within quarter of a mile of Poradihi, and it was affected by the same outbreak in April 1940. *A. subpictus* was the only species collected as larvæ. Adult mosquito collection: *A. sundaicus* 10, *A. subpictus* 93. Spleen rate 91 per cent.

Satpara is on an island separated only by a very narrow channel from a tongue projecting from the mainland at the north-eastern corner of the lake. There are numerous ricefields in the neighbourhood, protected by embankments from flooding by the lake water. Along the shore are a number of small pools dug by the local inhabitants for catching fish. Other brackish water collections are represented by a nala and two tanks near the shore. The only anopheline larvæ collected were those of *A. subpictus*. Adult mosquito collection: *A. subpictus* 108, *A. annularis* 5. Spleen rate 96 per cent.

Alupatna is a small village situated immediately to the south of Satpara. Three larvæ of *A. sundaicus* were collected from a pool in a nala close by, with salinity 350 parts per 100,000. Larvæ of *A. subpictus* were also collected. Adult mosquito collection: *A. subpictus*, 40. Spleen rate 97.

Nuagaon is a village with about 40 houses, situated 3 miles north-east of Satpara. There is a nala containing brackish water about half a mile from the village. The only larvæ collected were *A. subpictus*. Adult mosquito collection: *A. subpictus* 92, *A. annularis* 5. Spleen rate 98 per cent.

Sipakundapatna is on the same island as the last three villages, close to the channel which separates it from the mainland. Spleen rate 92 per cent.

Arakhakud is situated about 7 miles north-east of Sipakundapatna, on the margin of the channel connecting the lake with the Bay of Bengal, and as it lies immediately opposite the lake mouth it is, to all intents and purposes, a sea-side village. The soil around the village is sandy, and there are no collections of brackish water likely to provide favourable breeding places for *A. sundaicus*. Adult mosquito collection: *A. subpictus* 6. Spleen rate 11 per cent.

The lake itself in this area is affected by tidal action. Scanty floating Lyngbya and certain other algæ were seen near the shore in the vicinity of Satpara. Leaves of *Halophila ovata* were seen floating on the surface of the water and a sheet of *Salicornia* was observed near Garbai. The salinity of the lake in the neighbourhood of Satpara, Garbai and Tua varied from 2,025 to 2,250 parts per 100,000.

The extremely high spleen rates and the very large size of the average enlarged spleen in this area confirm the observations of Fry, and indicate that malaria is hyperendemic. Although no infected specimens were found, there seems no reason to doubt that *A. sundaicus*, which was present either in the adult or larval form in six of the villages visited, is the vector. Conditions round the villages, with the land embanked to protect the ricefields from flooding are very similar to those in the vicinity of the hyperendemic villages on the southern margin of the lake. The



case of Arakhakud, where there were no likely breeding places of *A. sundaicus*, and where the low spleen rate is in marked contrast with that in the other villages, lends further support to this view. Additional evidence is afforded by the fact that serious outbreaks of malaria commonly occur in the spring, as evidenced by the dispensary figures recorded at Garh Krishnaprasad over a number of years. The villages in this area no doubt constitute a permanent focus, from which *A. sundaicus* spreads to other villages round the lake margin whenever local conditions become especially favourable.

#### SURVEY OF VILLAGES AT THE NORTHERN END OF CHILKA LAKE.

Two visits were made to the area, the first in June 1941 and the second in December of the same year. The results of spleen examinations and collections of *A. sundaicus* are given in Table XVII.

In January 1941, no larvæ of *A. sundaicus* were found either in the lake itself or in the tanks and pools in the vicinity of the villages. The salinity in the lake was 1,500 parts per 100,000. The tanks were almost free from weeds, but some of the pools contained *Hydrilla*, *Najas* and *Ceratophyllum*.

In December 1941, the salinity in the lake was 350 parts per 100,000. There were scattered patches of algæ in it, but no anopheline larvæ were found. The tanks were again almost free from weeds, except that in some cases a growth of *Chlorella*, which is not usually associated with the breeding of *A. sundaicus*, was present. Larvæ of *A. sundaicus* were found in one pool out of six examined, all of which contained putrefying algæ and other weeds. The salinity of this pool was 60 parts per 100,000.

In the light of the other evidence put forward in the present article, the rise in the spleen rate at Bhusandpur from 26 to 73 in 6 months, together with the fact that adult specimens of *A. sundaicus* were captured there in December 1941, leaves little doubt that *A. sundaicus* is responsible for the outbreaks of malaria which occur in the villages lying at the northern end of the lake, just as in the case of those further south where routine investigations have been carried out during the last 3 years.

#### SURVEY OF VILLAGES SOUTH AND SOUTH-WEST OF GOPALPUR.

High spleen rates have been recorded in villages situated in the neighbourhood of Gopalpur Creek and outbreaks of malaria have occurred in Gopalpur itself. The investigations here recorded were carried out during May 1941 in order to find out how far *A. sundaicus* had infiltrated inland in the direction of the important town of Berhampore and whether Gopalpur Creek was the sole focus of malaria in this area.

There is an extensive body of water known as Gaungi Tampara, situated about 4 miles south-west of Gopalpur. Its maximum length and breadth during the rains are 4 and 2½ miles respectively. In the dry season, it is represented by a permanent water collection about 1 mile long lying between the villages of Kirtipur



and Hattipada and a number of isolated pools. In the rainy season, part of the excess water from the tampara passes into the sea over a narrow sand-bar near Markandi village, whilst the remainder finds an exit through a nala into the estuary of the Bahuda river. The salinity of the tampara on 15.v.41 was 25 parts per 100,000.

Investigations were carried out in certain villages situated between Gopalpur and Gaungi Tampara and between Gopalpur and Berhampore (Map 2). The results are shown in Table XVIII.

The low spleen rates recorded in the villages between Gopalpur and Gaungi Tampara indicate that the tampara itself is probably not a source of breeding of *A. sundaicus*. The high spleen rate at Nuagolabanda is almost certainly due to the breeding of *A. sundaicus* in a low-lying area which surrounds the village on three sides. The villagers stated that there had been an epidemic of malaria there in the autumn of 1940.

At the time of our visit in May 1941, an epidemic of malaria was actually in progress in Dura and the neighbouring hamlets of Bahadurpeta and Pathora. Dura is  $3\frac{1}{2}$  miles from the extreme upper end of Gopalpur Creek and  $2\frac{1}{2}$  miles from Berhampore.

The villagers stated that there was an outbreak of malaria at Ampua in the autumn of 1940 and that the epidemic at Dura started after this had subsided.

It is evident that *A. sundaicus* is the cause of the malaria outbreaks in this area just as it is in the vicinity of Chilka Lake, and it is probable that its primary breeding focus is provided by Gopalpur Creek and breeding places in its immediate vicinity.

#### RANGE OF INFILTRATION OF *A. SUNDAICUS* INLAND.

In order to determine the distance to which *A. sundaicus* infiltrates inland from the shore of Chilka Lake, a number of observations was carried out in certain groups of villages during the period November 1941 to March 1942 (Table XIX). The groups are placed in order from north to south.

It will be noted that the spleen rates decrease as the distance from the lake shore (or from the mouth of the Rushikulya river) increases, thus confirming the observations of Fry (*loc. cit.*) and Sarathy (*loc. cit.*). The density of *A. sundaicus*, as revealed in the adult mosquito collections, shows a correspondingly progressive fall. From November 1941 to March 1942, there was heavy breeding of *A. sundaicus* along the whole margin of the lake from Kumandol to Soran, i.e., opposite Groups I to IV inclusive. The villages farthest from the lake in which adult specimens of *A. sundaicus* were collected were Matapokra ( $5\frac{1}{2}$  miles) and Binjhala (5 miles), both in Group III. In the latter village, the single specimen captured appeared to be very recently hatched. In Group II, the range of infiltration observed was only 3 miles, but beyond Digitpara there are no villages in this neighbourhood, and there is a belt of thick forest which may act as a barrier.

Further south, the range of infiltration was more limited. There was sufficient local breeding of *A. sundaicus* in tanks at Kallikota (Group V) to account for the



number of adult specimens collected in this village. Similarly, in the spring epidemic of 1940, although larvæ of this species were present in the village tanks in Sabilia, adult specimens were very rare in the nearby hamlets of Bahadapalli and Gandapalli, and in the railway colony. In the Rushikulya area (Group VII), there was sufficient local breeding to account for the presence of the adult specimens of *A. sundaicus* caught at Boropalli and Ganjam.

Though the presence of *A. sundaicus* has been established at distances of approximately 6 miles from the lake shore, it is impossible to form an accurate estimate of the distance to which this species can infiltrate by direct flight. Larvæ were not found in the vicinity of any of the inland villages except Kallikota (2 miles from the lake shore) and Chikili (6 miles). It appears unlikely that the presence of adult specimens of *A. sundaicus* at Banpur (Group IV) was due to direct dispersion from the lake, because no specimens were caught at Sunakera, a village with a low spleen rate lying between Banpur and Lambodarpur, which is on the shore of the lake. The nearest point to the lake at which male specimens were captured was at Gangadharpur,  $2\frac{1}{2}$  miles from the lake margin.

There was a severe malaria epidemic at Chikili in 1937. No specimens of *A. sundaicus* appeared in the adult mosquito collections made in this village in October and November 1939 and in January and June 1940. There was another severe outbreak in the autumn of 1941, and 20 specimens of *A. sundaicus* were collected during a visit made in March 1942. There is no doubt that the recent epidemic was caused by this species, which was presumably also responsible for the outbreak in 1937.

#### RANGE OF INFILTRATION OF *A. SUNDAICUS* SOUTH OF CHILKA LAKE.

In June 1940, the presence of *A. sundaicus* was demonstrated at Gopalpur, 20 miles south of Chilka Lake. A reconnaissance survey of certain villages at the mouth of the Bahuda river, 33 miles south of the lake, made in October 1941, showed that the species was prevalent in this locality also. A second rapid survey was carried out in January 1942, with the object of finding out the probable southernmost limit to which *A. sundaicus* has infiltrated along the coast of the Bay of Bengal, the investigation being carried as far south as Konada, a village at the mouth of the Champavati-Nellimarla river 12 miles east of Vizianagram and 25 miles north of Vizagapatam (Map 2).

Owing to the pressure of other work, the investigation was limited to a spleen census of children supplemented by a search for adult mosquitoes, and a hunt for anopheline larvæ in such water collections as appeared favourable for the breeding of *A. sundaicus*.

The spleen rates in the various villages visited, together with data regarding larval and adult mosquito collections, are given in Table XX.

The furthest point south at which *A. sundaicus* was found was at Bavanapada, which is situated on the shore of an extreme backwater 81 miles south of Chilka Lake.



An abrupt change in the character of the aquatic vegetation found in the local water collections occurs at Calingapatam, 103 miles south of the Chilka Lake, the tanks to the south of this being almost entirely free from weeds. It seems possible that this may be a factor determining the southernmost limit of infiltration of *A. sundaiacus*.

It will be noted, however, that high spleen rates were encountered among the few children examined in certain cultivators' huts at Chintapalli, and at two villages lying 2 miles inland from Konada. *A. stephensi* and *A. culicifacies* were both captured at Konada, but any speculation as to the probable identity of the vector is valueless without evidence which can only be supplied by an extensive series of mosquito dissections.

### DISCUSSION.

The investigations here recorded have helped to fill many of the gaps in our knowledge of the epidemiology of malaria in the Orissa coastal belt. It has been shown that malaria is hyperendemic in the islands lying along the eastern margin of Chilka Lake, that the vector there is *A. sundaiacus*, and that this mosquito alone is probably responsible for the epidemics which occur from time to time in the villages situated round the lake margin and along the coast of the Bay of Bengal for many miles to the south. The limits of infiltration from the lake shore have been mapped out, and the extent to which its distribution extends southwards has been determined. It has been observed that the larvæ of this species are found most frequently and in greatest abundance in the presence of putrefying masses of algæ and weeds, and that, in all probability, the importance of salinity in this respect lies in the manner in which this affects the growth and life history of such vegetation.

The question as to whether the presence of *A. sundaiacus* in this area represents a recent invasion or not was raised by Senior White (*loc. cit.*). There is no evidence that any other species of *Anopheles* plays an appreciable part in malaria transmission in any of the localities visited during the investigations here recorded, and it seems highly improbable that the hyperendemic conditions now existing in the villages on the eastern margin of the lake should be the result of transmission by one vector, whilst conditions practically identical in 1912 should be caused by another. We have no doubt that the species was established there at the time of Fry's investigations. The question as to how long *A. sundaiacus* has been established on these islands prior to 1912 will probably never be answered.

In the light of the observations recorded in recent years, both in Lower Bengal and in the Orissa coastal belt, certain earlier records regarding the distribution of *A. sundaiacus* take on a new significance. In an article by Hodgson (1914) it was stated that *A. sundaiacus* (under its former name *A. ludlowi*) had been found infected by Horne in Madras City in the previous year, when there was a serious outbreak of malaria there. No figures were given either of the numbers collected or of those infected. *A. culicifacies* and *A. stephensi* were also met with but not found infected. Hodgson notes that, according to local opinion, malaria was quite a new feature in the history of the city. The outbreak commenced at the northern end and spleen



rates of 88 and 60 per cent were recorded in two localities in this area. In the light of our present knowledge, it seems quite possible that this was an epidemic due to an invasion by *A. sundaicus*.

It may be noted also that James (1914) records having reared specimens of *A. ludlowi* from rock pools along the sea shore in Colombo. The senior author cast doubt on this record (Covell, 1927b), but the events of the last decade suggest that it may well have been correct, particularly when James' standing as an authority on anopheline species is taken into account.

If the investigation here recorded has accomplished nothing else, it has at least served to emphasize the great importance and potentialities of *A. sundaicus* species as a malaria vector in India.

### CONTROL OF MALARIA IN THE ORISSA COASTAL BELT.

The investigations carried out in 1939 and 1940 on the breeding of *A. sundaicus* showed that this was closely associated with the presence of putrefying weeds and algæ, and that, when these settled down to the bottom of a breeding place after heavy rain, the breeding of this species was either greatly diminished or ceased altogether.

In 1941, experiments were carried out in certain tanks in Sabilia village to determine the effect and cost of mechanical removal of weeds. The tanks selected showed considerable individual variation as regards the type of aquatic vegetation present in them. Details of the experiments are shown in Table XXI. It will be seen that in 3 of the tanks, which it was found possible to maintain free from weeds and algæ, the breeding of *A. sundaicus* was completely arrested. In the other tank (2A), however, in which the only alga present was *Chætomorpha*, the breeding of *A. sundaicus* continued, though on a greatly reduced scale. It was found impossible to keep this tank free from algæ by hand removal, and therefore on 16.iii.41, copper sulphate was added in the proportion of 1/420,000. This had no appreciable result, but a further application in the proportion of 1/60,000 on 22.iii.41 effectively checked the growth of algæ, and after this the breeding of *A. sundaicus* no longer persisted. The effect of removal of weeds was also studied in certain tanks at Dubrakudi and Balugaon and in a borrowpit near the railway station at Humma. In all cases, the removal of the weeds (*Ceratophyllum* and *Spirogyra*) was followed by the cessation of breeding of *A. sundaicus*.

Further experiments were carried out in the period September 1941 to March 1942, in the area extending from Konoka to Rambha Bay, where the chief breeding places of *A. sundaicus* are tanks, borrowpits, swamps and pools near the shore of Chilka Lake. Breeding of this species in the lake itself was negligible in this area during the period of the investigation. The operations were carried out in 5 villages, viz., Sabilia, Rambha, Konoka, Matkona and Diadei. Five other villages (Redika Bahadapalli, Hadi Bahadapalli, Gandapalli, Gopinathpur and Michinpatna) in the same area are sufficiently near to have received some benefit from the control operations. Observations were carried out in Kesopur (north of the controlled area) and the Proyagi group of villages (south of the controlled area) during the



same period for comparison purposes. The average daily collection of *A. sundaicus* per week in the controlled area and in the comparison villages is given in Table XXII.

The staff employed in this area consisted of 1 mate and 4 coolies and the breeding places were cleaned twice a week. The working hours were 7 a.m. to 12 noon and 3 p.m. to 5 p.m. The principle aimed at was to remove floating weeds as far as possible so as to prevent putrefaction. This was done by means of nets, of  $\frac{3}{4}$ -inch mesh (linear measurement), which was found the most convenient size of aperture for the work. Total removal of weeds was impracticable and was not attempted.

In the area where control operations were carried out, no evidence of active transmission of malaria was observed during this period. At Rambha, there was a fall in the spleen rate from 47 per cent in August 1941 to 27 per cent in March 1942. At Sabilia, there was a slight fall from 72 to 60 per cent. At Konoka, there was no appreciable fall in the spleen rate, but the average enlarged spleen decreased from 8.7 cm. (A-U measurement) in April 1941 to 9.8 cm. in March 1942. At Diadei, there was a slight fall in the spleen rate from 100 per cent in August 1941 to 93 per cent in March 1942, with a decrease in the average enlarged spleen from 7.8 cm. to 8.2 cm. during the same period. At Kesopur (uncontrolled), the spleen rate was unchanged, but the size of the average enlarged spleen increased from 8.6 in August 1941 to 8.1 in March 1942. At Proyagi, the average enlarged spleen increased from 9.0 cm. in December 1941 to 7.7 cm. in March 1942. Parasite rates were considerably higher at Kesopur than at Sabilia.

These figures are not absolutely conclusive, but taken all together the results are distinctly encouraging, considering the limited period during which control operations were in progress. The inhibiting effect of weed removal on the breeding of *A. sundaicus* was demonstrated beyond doubt.

The cost of the labour employed during the 6 months from September 1941 to March 1942 was Rs. 328. The mate, who was also trained as an insect collector, received Rs. 20 p.m., and the coolies Rs. 9 each. The cost of equipment (ropes, nets, copper sulphate, etc.) was approximately Rs. 8. If the work had been continued to the end of April, which would have been necessary in an antimalaria campaign, the total cost would have been about Rs. 410. As the total population protected is in the neighbourhood of 9,000, the annual cost per head of population would be less than 9 pies ( $\frac{3}{4}$  anna). This does not take into account the cost of supervision, which is an indispensable item in any antimalaria campaign. But if the campaign were undertaken on a large scale, one responsible officer would be able to exercise supervision over an extensive stretch of the Orissa coastline.

In villages like Konoka, Sabilia and Rambha, where outbreaks of malaria are due to local breeding of *A. sundaicus* in certain pools and tanks, the removal of weeds by hand offers a good prospect of success. Where the lake itself is the main source of breeding, the problem is much more difficult. If the salinity could be raised to above 1,000 parts per 100,000 by admitting sea water to the lake, the breeding of *A. sundaicus* would cease. But even if this could be done, the cost of



the work, and particularly of its maintenance, would be altogether prohibitive. The difficulties likely to be encountered in any attempt to alter physical conditions in the Chilka Lake are stressed in the report of the Orissa Flood Committee (1929).

Removal of the weeds by hand at first appeared impracticable, but an experiment in this direction was commenced in March 1942 at Bolabandh in collaboration with the District Health Officer, Puri. This experiment was still in progress when the research unit was withdrawn, and it is hoped that it will be continued by the Government of Orissa until sufficient data have been accumulated to demonstrate its value and cost. The local inhabitants evinced great interest in the work, and actually contributed a sum of money towards the cost. It seems possible that belts of weeds could be removed over an extensive area by a wire net stretched between two boats. It was unfortunate that circumstances brought about by the war necessitated the withdrawal of the research unit before further experiments with this method of control could be carried out.

As an additional measure to be employed in any village in which an outbreak occurs, the method of spray-killing adult mosquitoes with pyrethrum insecticide as practised in Delhi (Covell *et al.*, 1938), Madras (Russell and Knipe, 1939; 1940; 1941), Assam (Viswanathan, 1941; 1942) and elsewhere offers the best prospect of success. It is the only method which can have an immediate mitigating effect on the progress of an epidemic, and should, in all cases, be put into effect with the utmost vigour until effective control of breeding places can be secured.

The breeding of *A. sundanicus* in ricefields has been observed only when the crop is scanty and short stemmed. There is a variety of rice in this neighbourhood which grows densely and has long stems, and it is suggested that this variety should be grown in those fields which are close to the shore of a lake or creek. This is a matter in which the advice of an agricultural expert is essential.

At Chatrapur the chief breeding places of *A. sundanicus* are the tampara and certain tanks and borrowpits. It is suggested that the tampara be drained by removing the dam at its northern end and the land exposed put under rice cultivation, a long stemmed and densely growing variety of rice being selected for this purpose. The removal of weeds from the tampara is the only alternative measure, but the cost of this would probably be prohibitive.

For the Gopalpur area the following measures are suggested :—

- (i) Removal of vegetation from tanks and pools in and around villages.
- (ii) Filling in of borrowpits and prohibition of further excavation of earth except in that part of the creek subject to regular flooding by the spring tides. Any pits dug should be so constructed that the water will drain out of them into the creek when the tide falls.
- (iii) Strengthening of the existing embankments so as to prevent the flooding of ricefields by saline water.



(iv) Construction of a dam across the mouth of Kandla Nala to prevent the entrance of saline water, similar to that which already exists at the junction between the old navigation canal and the creek. The height of the latter dam should also be raised so as to prevent the entrance of saline water.

(v) The mouth of Gopalpur Creek should be kept open throughout the year.

Malaria problems in Orissa are many and varied and the high incidence of the disease in most parts of the province is one of the principal barriers in the path of its successful economic development. In the past, the control of malaria in the province has been hampered by the absence of a permanent malaria organization. A small staff under a special malaria officer was sanctioned in 1939 for a period of 3 years, and this has now been placed on a permanent basis. This is a step in the right direction, but it is to be hoped that the organization will be expanded in the near future. To be effective, antimalaria schemes must be carried out regularly from year to year like any other public health measure, and they need expert supervision and direction at all times. Continuity of service in the malaria organization is, therefore, most essential, and the salaries provided should be on a scale sufficiently liberal to render its officers content to remain in their posts so that they may not be continually on the look-out for more lucrative employment. The status of the officer-in-charge of the organization should be at least equal to that of an Assistant Director of Public Health.

### SUMMARY.

1. Investigations regarding malaria conditions in the coastal belt of Orissa Province were carried out during the period April 1939 to March 1942. Detailed routine observations were made in the area extending from Chatrapur, 16 miles south of Chilka Lake, to Sana Nairi, half-way along its western margin, throughout this period, and special expeditions to investigate particular problems were made to a number of other villages in the vicinity of the lake and in the coastal belt as far south as Vizianagram.

2. The distribution of malaria in this area is patchy and irregular. Hyperendemic conditions prevail in certain villages, and epidemics of a serious nature occur from time to time either in the spring or autumn months.

3. The malaria vector throughout the region is *A. sundaicus*. There is no evidence that any other species plays any part in the transmission of the disease.

4. *A. sundaicus* breeds in Chilka Lake itself when conditions are favourable, and also in tanks, pools, swamps and ricefields subject to flooding by saline water.

5. The furthest distance inland at which *A. sundaicus* was found was 6 miles from the shore of Chilka Lake. The most southern point at which it was found was at Bavanapada, 81 miles south of the Chilka Lake.

6. The bearing of the investigations made in recent years on the validity of certain earlier records regarding the distribution of *A. sundaicus* is discussed.



7. The chief factor favouring the breeding of *A. sundanicus* is the presence of putrefying algæ and other weeds. The optimum range of salinity in this area is from 600 to 800 parts per 100,000, but salinity probably only operates in so far as it affects the growth of the weeds.

8. Removal of weeds from breeding places is followed by a cessation of breeding of *A. sundanicus*. Experiments carried out in certain villages indicate that this is the most promising method of controlling malaria in the Orissa coastal belt.

9. The spray-killing of adult mosquitoes with pyrethrum insecticide is recommended as the most effective emergency measure to deal with epidemics of malaria in this area.

#### ACKNOWLEDGMENTS.

We wish to express our thanks to Major R. Senior White, Malariologist, Bengal-Nagpur Railway, for his assistance on many occasions and for placing the data collected by him and his assistants at our disposal and to Mr. Bansidhar Parija, Ravenshaw College, Cuttack, for his assistance in identifying various types of aquatic vegetation.

#### REFERENCES.

- ANNANDALE, N., and KEMP, S. (1915) .. Fauna of Chilka Lake—aquatic insects. *Mem. Ind. Mus.*, **5**, pp. 1-20.
- COVELL, G. (1927a) .. *Report of an inquiry into malarial conditions in the Andamans*. Delhi: Govt. of India Press.
- Idem* (1927b) .. The distribution of anopheline mosquitoes in India and Ceylon. *Ind. Med. Res. Mem.*, **5**, p. 45.
- COVELL, G., MULLIGAN, H. W., and AFRIDI, M. K. (1938). An attempt to control malaria by the destruction of adult mosquitoes with insecticidal sprays. *J. Mal. Inst. Ind.*, **1**, 1, pp. 105-113.
- FRY, A. B. (1912) .. *First report on malaria in Bengal*. 33 pp. Calcutta: Bengal Sectt. Book Depot.
- HODGSON, E. C. (1914) .. A preliminary note on malaria in Madras City. *Ind. J. Med. Res.*, **1**, 4, pp. 702-706.
- JAMES, S. P. (1914) .. Summary of a year's mosquito work in Colombo. *Ibid.*, **2**, 1, pp. 227-267.
- ORISSA FLOOD COMMITTEE, 1928 (1929) .. *Report*. Patna: Govt. Press.
- RUSSELL, P. F., and KNIPE, F. W. (1939) Malaria control by spray-killing adult mosquitoes. First season's results. *J. Mal. Inst. Ind.*, **2**, 3, pp. 229-237.
- Idem* (1940) .. Malaria control by spray-killing adult mosquitoes. Second season's results. *Ibid.*, **3**, 4, pp. 531-541.
- Idem* (1941) .. Malaria control by spray-killing adult mosquitoes. Third season's results. *Ibid.*, **4**, 2, pp. 181-197.
- SARATHY, M. N. P. (1932) .. Observations on malaria in Puri District, Orissa. *Ind. Med. Gaz.*, **67**, pp. 254-259.
- SENIOR WHITE, R. (1937) .. The presence of *A. sundanicus* (*ludlowi*) on the Chilka Lake. *Ibid.*, **72**, pp. 361-363.
- SENIOR WHITE, R., and ADHIKARI, A. K. (1939). On malaria transmission around the Chilka Lake. *J. Mal. Inst. Ind.*, **2**, 4, pp. 395-423.
- SEWELL, R. B. S., and ANNANDALE, N. (1922). The hydrography and invertebrate fauna of Rambha Bay. *Mem. Ind. Mus.*, **5**, 10, p. 667.
- SINTON, J. A., and KEHAR, N. D. (1930) .. A field method for estimation of the salinity of water in mosquito breeding places. *Ind. J. Med. Res.*, **18**, pp. 199-201.



- VISWANATHAN, D. K. (1941) .. Experimental malaria control in a hyperendemic tea garden in Upper Assam by the use of Pyroicide 20 as an insecticidal spray. *J. Mal. Inst. Ind.*, 4, 1, pp. 139-152.
- Idem* (1942) .. Malaria control by spray-killing adult anophelines. Second season's results. *Ibid.*, 4, 3, pp. 393-404.

## APPENDIX.

The following information regarding malaria conditions at certain railway stations on the B. N. Railway from Bhusandpur to Humma during the period November 1941 to April 1942 has been kindly placed at our disposal by Major R. Senior White.

(1) There is a considerable difference between the proportion of *A. sundaicus* resting in houses and cowsheds during different seasons of the year as shown below :—

	GANGADHARPUR.		KALLIKOTA.	
	House.	Cowshed.	House.	Cowshed.
November ..	30	38	15	16
December ..	101	100	8	14
January ..	153	156	28	25
February ..	81	50	24	22
March ..	120	19	46	11
April ..	46	4	12	6

So long as the weather is cool the numbers found in the two types of resting places are about equal, but when it becomes warmer the species does not appear to rest in cowsheds to the same extent.

(2) The peak of malaria incidence was October at Rambha and November at Bhusandpur, December at Kallikota, Balugaon and Gangadharpur, and January at Kalupara Ghat and Kuhuri. There was no sign of a regular progression from south to north.

(3) Of the positive blood films 66 per cent were *P. falciparum* and the remainder *P. vivax*. No *P. malariae* were encountered.

(4) Malaria control by spray-killing adult mosquitoes was practised in all stations, but the results were disappointing. It may be that the mosquitoes obtain their first, and often infected, feed in the lake-side villages and are infective by the time they have infiltrated to the railway. They may thus infect the railway personnel during the night following their arrival.



TABLE I.

*Salinity figures, Chilka Lake (parts per 100,000), April 1939 to March 1942.*

Month.	Sana Nairi.				Balugaon.				Kesapur.			
	1939.	1940.	1941.	1942.	1939.	1940.	1941.	1942.	1939.	1940.	1941.	1942.
January	..	750	525	525	..	700	575	675	..	825	750	850
February	..	750	800	575	..	775	775	725	..	850	850	950
March	..	..	850	725	..	750	875	775	..	..	900	850
April	..	..	900	..	..	775	875	..	..	..	950	..
May	..	1,500	1,250	..	..	1,000	1,550	..	..	..	1,150	..
June	1,250	875	1,275	..	1,300	650	1,325	..	1,200	825	1,400	..
July	750	75	775	..	950	75	1,475	..	1,450	750	1,175	..
August	625	25	425	..	600	45	500	..	1,450	874	800	..
September	..	25	400	..	40	..	..	..	950	860	800	..
October	250	285	350	..	425	350	150	..	950	885	750	..
November	425	550	250	..	700	350	450	..	750	885	750	..
December	625	500	275	..	325	500	525	..	650	850	650	..

Month.	Konoka.				Sabalia.				Dubrakudi.				Chilka Canal.			
	1939.	1940.	1941.	1942.	1939.	1940.	1941.	1942.	1939.	1940.	1941.	1942.	1939.	1940.	1941.	1942.
January	..	950	850	825	..	900	650	700	..	850	1,050	..	..	1,050	1,675	..
February	..	950	850	..	..	825	875	825	..	1,100	1,050	..	..	2,075	1,625	..
March	..	1,000	875	1,150	..	1,325	875	850	..	1,250	1,050	1,225	..	1,800	1,050	..
April	..	1,000	..	..	925	1,375	875	..	..	1,275	1,050	..	..	1,400	1,050	..
May	1,225	1,150	1,075	..	1,050	1,450	1,175	..	..	1,325	1,275	..	700	1,850	1,350	..
June	1,450	825	1,375	..	1,200	825	1,325	..	1,175	1,000	1,250	..	1,500	1,925	1,250	..
July	1,575	775	1,450	..	1,375	969	1,375	..	1,300	875	1,625	..	1,550	875	1,700	..
August	1,450	1,025	1,450	..	1,450	692	1,350	..	1,250	1,000	1,150	..	1,250	1,100	1,300	..
September	500	1,075	..	..	1,125	1,012	1,050	..	1,175	850	1,200	..	1,175	850	1,200	..
October	750	1,050	725	..	950	825	800	..	1,000	1,250	..	..	1,000	1,250	..	..
November	900	1,075	800	..	850	800	850	..	775	975	850	..	775	1,550	950	..
December	850	775	..	..	850	625	775	..	850	1,050	850	..	850	1,125	1,000	..



TABLE II.

*Average weekly maximum and minimum temperature and relative humidity recorded at Rambha, July 1939 to March 1942.*

Week ending			AVERAGE TEMPERATURE.		AVERAGE RELATIVE HUMIDITY, PER CENT.	
			Maximum, °F.	Minimum, °F.	8 a.m.	4 p.m.
<b>1939.</b>						
July	23	..	88·3	76·4	..	..
"	30	..	91·0	79·3	..	..
August	6	..	93·1	81·1	..	..
"	13	..	90·0	79·3	74·0	..
"	20	..	87·9	78·0	63·2	..
"	27	..	89·3	78·7	55·4	..
September	3	..	90·1	79·0	67·3	..
"	10	..	89·6	78·4	72·5	..
"	17	..	87·7	76·1	65·4	..
"	24	..	88·9	77·4	66·7	..
October	1	..	92·1	76·6	62·2	..
"	8	..	90·6	76·0	66·7	..
"	15	..	88·3	77·0	67·9	..
"	22	..	85·3	75·4	70·6	..
"	29	..	84·9	74·7	67·6	..
November	5	..	85·4	70·6	56·3	..
"	12	..	84·6	68·3	56·5	..
"	19	..	81·1	68·9	64·7	..
"	26	..	78·9	63·9	47·9	..
December	3	..	79·9	63·4	59·4	..
"	10	..	77·3	67·1	48·3	..
"	17	..	79·7	67·0	61·0	..
"	24	..	75·9	54·9	52·9	..
"	31	..	77·9	59·6	53·4	..



TABLE II—*contd.*

Week ending	AVERAGE TEMPERATURE.		AVERAGE RELATIVE HUMIDITY, PER CENT.	
	Maximum, °F.	Minimum, °F.	8 a.m.	4 p.m.
<b>1940.</b>				
January 7 ..	75·3	57·0	48·4	..
„ 14 ..	77·1	53·9	45·6	..
„ 21 ..	81·7	57·3	58·1	..
„ 28 ..	79·4	60·4	61·4	..
February 4 ..	84·4	60·7	51·7	..
„ 11 ..	82·4	69·0	67·7	..
„ 18 ..	79·7	63·4	53·3	..
„ 25 ..	85·7	65·1	54·0	..
March 3 ..	87·4	70·9	54·7	..
„ 10 ..	84·7	74·1	64·3	..
„ 17 ..	86·6	72·3	65·7	50·8
„ 24 ..	86·7	73·1	57·6	56·6
„ 31 ..	89·1	71·6	68·5	71·9
April 7 ..	94·3	77·1	61·6	60·6
„ 14 ..	96·1	75·9	53·2	55·3
„ 21 ..	94·3	77·0	55·4	44·2
„ 28 ..	94·3	76·6	45·9	44·9
May 5 ..	96·0	81·9	49·7	47·5
„ 12 ..	96·4	83·1	56·3	52·5
„ 19 ..	93·1	82·5	56·9	52·2
„ 26 ..	91·0	78·1	63·2	58·4
June 2 ..	92·0	75·9	57·8	57·4
„ 9 ..	92·4	80·7	51·8	53·3
„ 16 ..	92·7	82·7	58·7	56·9
„ 23 ..	89·9	78·7	60·6	60·9
„ 30 ..	86·0	79·4	63·4	58·9



TABLE II—*contd.*

Week ending			AVERAGE TEMPERATURE.		AVERAGE RELATIVE HUMIDITY, PER CENT.	
			Maximum, °F.	Minimum, °F.	8 a.m.	4 p.m.
<b>1940.</b>						
July	7	..	87·1	78·6	57·6	61·0
"	14	..	87·0	78·0	55·3	56·1
"	21	..	87·9	79·0	63·9	59·9
"	28	..	87·3	77·4	63·6	61·3
August	4	..	88·9	79·6	62·6	58·8
"	11	..	90·6	78·0	57·0	60·4
"	18	..	84·7	77·3	68·6	63·7
"	25	..	87·6	78·1	60·9	63·6
September	1	..	91·3	79·9	55·4	54·0
"	8	..	91·0	78·0	63·8	57·7
"	15	..	91·0	76·3	61·4	60·7
"	22	..	89·3	77·7	60·9	57·4
"	29	..	90·9	76·4	61·6	55·6
October	6	..	91·7	76·3	53·4	39·1
"	13	..	88·3	74·9	60·6	53·5
"	20	..	88·4	74·9	64·2	57·8
"	27	..	91·1	71·4	57·9	48·4
November	3	..	88·3	73·0	66·7	49·3
"	10	..	87·1	67·1	54·3	39·0
"	17	..	83·4	70·7	50·7	44·7
"	24	..	84·6	73·3	56·0	55·8
December	1	..	86·1	71·1	58·3	50·3
"	8	..	84·3	66·0	50·4	35·0
"	15	..	81·7	64·4	48·3	40·3
"	22	..	81·4	66·4	51·6	40·1
"	29	..	76·3	55·3	42·7	31·3



TABLE II—*contd.*

Week ending			AVERAGE TEMPERATURE.		AVERAGE RELATIVE HUMIDITY. PER CENT.	
			Maximum, °F.	Minimum, °F.	8 a.m.	4 p.m.
<b>1941.</b>						
January	5	..	78.9	57.3	42.0	33.0
"	12	..	82.8	64.3	57.7	44.3
"	19	..	81.4	64.3	52.7	44.4
"	26	..	85.4	67.7	64.4	54.1
February	2	..	81.8	68.6	59.7	40.7
"	9	..	81.6	61.1	41.0	31.7
"	16	..	85.4	69.3	51.7	40.1
"	23	..	88.6	69.7	58.7	43.5
March	2	..	89.8	68.0	46.2	33.2
"	9	..	92.1	68.1	38.2	27.3
"	16	..	92.6	73.7	42.4	37.4
"	23	..	94.4	73.6	40.5	37.1
"	30	..	94.9	76.6	39.1	33.6
April	6	..	96.1	73.9	34.3	35.9
"	13	..	95.1	77.4	50.2	43.6
"	20	..	98.6	81.0	52.9	45.3
"	27	..	95.1	79.6	43.4	39.9
May	4	..	95.4	81.6	41.9	41.4
"	11	..	95.6	82.4	35.0	35.4
"	18	..	97.9	81.7	34.4	32.5
"	25	..	96.9	81.9	39.0	35.4
June	1	..	97.6	84.4	40.4	36.2
"	8	..	92.4	80.6	41.3	35.4
"	15	..	93.7	80.1	72.3	69.7
"	22	..	95.7	80.9	77.7	73.9
"	29	..	90.6	78.7	83.6	79.7
July	6	..	87.6	79.0	81.3	78.2
"	13	..	83.7	74.9	80.4	75.9
"	20	..	94.0	79.7	72.4	67.6
"	27	..	93.4	79.6	75.5	70.9



TABLE II—*concl'd.*

Week ending			AVERAGE TEMPERATURE.		AVERAGE RELATIVE HUMIDITY, PER CENT.	
			Maximum, °F.	Minimum, °F.	8 a.m.	4 p.m.
<b>1941.</b>						
August	3	..	89·3	78·0	82·1	78·6
"	10	..	89·3	80·1	80·9	78·1
"	17	..	91·0	78·3	80·0	76·6
"	24	..	92·0	80·6	72·4	67·3
"	31	..	91·4	79·7	78·4	67·7
September	7	..	91·4	77·7	78·2	73·8
"	14	..	87·6	77·6	82·1	74·1
"	21	..	89·7	78·1	77·8	79·3
"	28	..	93·7	77·5	76·6	70·9
October	5	..	87·6	76·3	71·8	74·1
"	12	..	85·6	76·7	80·1	75·8
"	19	..	88·6	76·7	72·6	64·4
"	26	..	88·6	75·0	76·7	69·1
November	2	..	86·9	71·0	70·7	57·8
"	9	..	87·1	68·4	74·1	55·1
"	16	..	84·0	67·1	66·1	53·5
"	23	..	78·6	66·6	78·1	67·0
"	30	..	79·0	60·7	71·0	45·6
December	7	..	79·4	64·0	65·4	63·0
"	14	..	80·7	65·7	68·1	61·7
"	21	..	80·1	64·0	78·1	66·9
"	28	..	83·7	63·1	67·6	62·1
<b>1942.</b>						
January	4	..	82·1	63·9	67·6	52·4
"	11	..	80·1	62·4	70·4	56·6
"	18	..	81·6	62·3	75·1	55·7
"	25	..	78·7	59·3	70·4	50·7
February	1	..	80·6	60·4	70·1	52·0
"	8	..	82·7	63·6	75·0	59·7
"	15	..	86·4	63·0	70·6	55·3
"	22	..	87·3	68·7	85·7	70·9
March	1	..	88·7	72·4	79·7	63·5
"	8	..	91·1	70·1	76·6	77·6
"	15	..	89·9	71·7	75·4	67·5
"	22	..	91·6	69·4	75·9	..



TABLE III.

*Monthly rainfall in inches recorded at Rambha, January 1929 to March 1942.*

Month.	1929.	1930.	1931.	1932.	1933.	1934.	1935.	1936.	1937.	1938.	1939.	1940.	1941.	1942.
January	0.30	0.00	0.00	0.00	0.00	0.00	0.00	1.12	0.00	0.90	0.00	0.00	0.56	0.00
February	1.16	1.38	0.85	0.50	0.00	0.00	0.00	4.98	7.70	0.00	0.00	2.65	0.00	0.73
March	0.00	0.10	0.00	0.00	0.00	0.00	0.00	0.00	0.50	2.00	0.60	3.46	0.03	0.00
April	0.00	2.20	0.00	0.00	0.00	0.00	1.53	0.00	1.10	0.00	0.00	0.16	0.55	..
May	0.73	0.94	0.45	3.30	1.50	0.90	0.00	6.60	2.80	6.65	0.70	12.86	0.21	..
June	4.40	8.10	2.25	0.00	3.64	2.19	0.65	8.92	3.14	5.96	6.24	10.94	6.97	..
July	4.55	9.80	0.60	9.40	6.80	5.90	9.81	10.45	7.80	5.57	6.39	8.76	8.43	..
August	10.33	7.23	8.70	5.90	10.18	6.14	2.32	8.67	7.30	5.28	9.09	11.01	3.64	..
September	9.11	9.16	10.30	5.15	11.85	5.98	9.52	7.50	2.50	10.30	6.64	6.12	7.05	..
October	17.98	3.63	14.70	5.50	10.70	2.70	2.02	8.35	4.25	7.80	7.67	4.26	7.33	..
November	0.00	11.60	0.00	12.30	1.90	0.73	0.00	0.00	0.00	0.80	1.00	0.15	1.86	..
December	0.00	0.00	0.00	0.00	0.31	0.00	0.00	0.00	0.00	0.00	0.26	0.00	0.00	..
TOTAL ..	48.56	54.16	37.85	42.05	46.88	24.54	25.85	56.59	37.09	45.26	38.59	60.37	36.63	0.73

TABLE IV.

*Cases of malaria treated at Kalikota dispensary, January 1931 to February 1942.*

Month.	1931.	1932.	1933.	1934.	1935.	1936.	1937.	1938.	1939.	1940.	1941.	1942.
January	..	67	60	76	74	99	219	118	208	70	366	224
February	..	84	111	104	75	100	139	244	298	59	242	185
March	..	143	266	227	40	106	409	1,018	618	118	265	..
April	..	137	155	281	131	135	752	920	217	156	206	..
May	..	138	110	132	107	72	588	224	221	112	1,172	..
June	..	95	83	113	78	68	248	208	63	101	137	..
July	..	84	93	122	93	133	247	185	46	145	192	..
August	..	97	60	131	96	236	275	196	104	158	113	..
September	..	78	116	110	72	114	263	139	78	111	110	..
October	..	115	127	132	70	57	196	49	113	174	148	..
November	..	98	34	83	96	162	381	54	60	582	135	..
December	..	58	54	57	70	173	402	184	41	591	146	..



TABLE V.

*Cases of malaria treated at Rambha dispensary, January 1935 to February 1942.*

Month.	1935.	1936.	1937.	1938.	1939.	1940.	1941.	1942.
January	112	26	192	242	227	86	149	62
February	102	51	36	306	157	68	131	60
March	109	169	189	265	241	110	125	..
April	112	559	741	250	202	219	137	..
May	92	328	686	184	154	216	108	..
June	101	157	268	127	94	160	122	..
July	61	113	179	103	156	146	78	..
August	47	128	223	92	222	135	94	..
September	55	113	256	94	125	133	91	..
October	68	90	412	120	127	236	82	..
November	35	227	630	138	146	370	67	..
December	38	486	680	306	118	340	97	..

TABLE VI.

*Cases of malaria treated at Ganjam dispensary, January 1931 to February 1942.*

Month.	1931.	1932.	1933.	1934.	1935.	1936.	1937.	1938.	1939.	1940.	1941.	1942.
January	131	62	45	17	64	23	135	258	240	274	430	124
February	180	74	34	29	27	46	359	209	248	220	414	118
March	200	83	30	54	70	73	726	236	254	383	532	..
April	206	72	37	69	97	120	1,022	418	251	343	441	..
May	185	62	44	29	40	81	876	392	194	339	597	..
June	91	80	64	52	48	71	689	336	192	609	464	..
July	139	82	98	41	39	58	794	330	250	494	301	..
August	184	120	58	74	48	67	673	387	333	500	136	..
September	192	91	47	99	32	66	319	503	319	531	135	..
October	57	43	56	99	30	48	266	306	467	372	193	..
November	106	40	26	81	26	119	321	189	669	414	237	..
December	73	30	37	34	15	212	356	210	438	620	133	..



TABLE VII.  
Cases of malaria treated at Government Hospital, Chattrapur. January 1931 to February 1942.

Month.	1931.	1932.	1933.	1934.	1935.	1936.	1937.	1938.	1939.	1940.	1941.	1942.
January	..	235	81	95	180	111	108	300	367	218	282	195
February	..	101	91	83	115	142	96	310	295	209	152	154
March	..	137	148	243	139	194	193	352	400	177	190	..
April	..	335	133	154	151	118	395	359	327	217	221	..
May	..	220	135	142	116	100	343	356	227	221	236	..
June	..	406	185	113	122	54	387	574	161	396	218	..
July	..	303	132	176	103	62	916	815	192	354	223	..
August	..	558	184	166	119	59	716	586	206	373	307	..
September	..	387	222	208	107	57	511	521	241	261	268	..
October	..	273	142	206	93	55	343	460	264	242	224	..
November	..	94	133	139	100	66	337	230	248	542*	126	..
December	..	139	118	131	95	84	302	240	227	734*	135	..

\* This increase of cases appears to be due to inclusion of patients from surrounding villages. The spleen rate does not show any marked rise.

TABLE VIII.  
Cases of malaria treated at Gopalpur dispensary. January 1931 to January 1942.

Month.	1931.	1932.	1933.	1934.	1935.	1936.	1937.	1938.	1939.	1940.	1941.	1942.
January	..	257	129	14	24	31	111	125	236	168	311	112
February	..	169	60	33	16	71	125	122	169	147	260	..
March	..	108	82	47	44	91	285	204	196	133	189	..
April	..	157	117	18	39	82	106	355	139	116	148	..
May	..	168	102	32	35	99	112	313	150	97	127	..
June	..	161	147	24	49	136	211	213	136	167	130	..
July	..	222	76	29	32	80	543	225	160	220	250	..
August	..	652	100	38	29	154	770	235	179	237	356	..
September	..	564	90	53	43	123	607	281	181	217	272	..
October	..	320	104	42	27	108	212	316	188	211	217	..
November	..	267	71	32	23	112	137	169	173	260	163	..
December	..	103	51	37	15	101	216	272	168	355	175	..



TABLE

Results of routine spleen and

Name of village.		1939.					
		May.	June.	July.	Aug.	Sept.	Oct.
Soran .. (Chilka Lake west).	Number of spleen examinations ..	60	..	..	80	..	90
	Spleen rate per cent ..	77	..	..	66	..	63
	Average enlarged spleen (cm. from umbilicus).	7.7	..	..	8.4	..	8.7
	Number of blood examinations ..	50	..	..	50	..	50
	Parasite rate per cent ..	48	..	..	26	..	14
	Gametocyte rate per cent ..	32	..	..	16	..	8
	Average positive parasite count per c.mm. of blood.	1,806	..	..	768	..	1,496
	Species of parasite	Asexual ..	..	..	..	..	..
		Sexual ..	253	..	244	..	218
		<i>P. falciparum</i> ..	8	..	7	..	1
		<i>P. vivax</i> ..	17	..	8	..	7
		<i>P. malariae</i> ..	0	..	0	..	0
Sana Nairi .. (Chilka Lake west).	Number of spleen examinations ..	85	100	110	100	100	100
	Spleen rate per cent ..	65	67	71	69	49	52
	Average enlarged spleen (cm. from umbilicus).	8.4	7.6	8.7	8.5	8.6	9.0
	Number of blood examinations ..	..	50	50	50	50	50
	Parasite rate per cent ..	..	12	20	12	6	4
	Gametocyte rate per cent ..	..	8	8	10	2	2
	Average positive parasite count per c.mm. of blood.	..	508	754	242	200	625
	Species of parasite	Asexual ..	..	..	..	..	..
		Sexual ..	243	164	36	100	400
		<i>P. falciparum</i> ..	1	9	3	1	0
		<i>P. vivax</i> ..	5	1	3	2	2
		<i>P. malariae</i> ..	0	0	0	0	0
Balugaon .. (Chilka Lake west).	Number of spleen examinations ..	100	100	120	125	125	101
	Spleen rate per cent ..	36	23	18	14	10	10
	Average enlarged spleen (cm. from umbilicus).	8.5	9.6	9.6	8.5	9.3	10.2
	Number of blood examinations ..	100	50	50	50	50	50
	Parasite rate per cent ..	8	6	2	2	2	2
	Gametocyte rate per cent ..	5	4	0	2	2	0
	Average positive parasite count per c.mm. of blood.	428	158	20	400	20	40
	Species of parasite	Asexual ..	110	50	60	40	0
		Sexual ..	7	2	1	0	1
		<i>P. falciparum</i> ..	2	1	0	1	0
		<i>P. vivax</i> ..	0	0	0	0	0
Kesopur .. (Chilka Lake west).	Number of spleen examinations ..	..	90	90	90	90	80
	Spleen rate per cent ..	..	88	84	72	60	64
	Average enlarged spleen (cm. from umbilicus).	..	8.2	8.3	7.9	8.7	9.2
	Number of blood examinations ..	..	50	50	50	50	50
	Parasite rate per cent ..	..	6	10	8	8	2
	Gametocyte rate per cent ..	..	6	10	2	4	0
	Average positive parasite count per c.mm. of blood.	..	233	194	1,278	45	200
	Species of parasite	Asexual ..	..	..	..	..	..
		Sexual ..	100	100	75	20	0
		<i>P. falciparum</i> ..	3	1	4	2	1
		<i>P. vivax</i> ..	0	4	0	3	0
		<i>P. malariae</i> ..	0	0	0	0	0



## IX.

## blood examinations of children.

1940.													
Nov.	Dec.	Jan.	Feb.	March.	April.	May.	June.	July.	Aug.	Sept.	Oct.	Nov.	Dec.
100 63 8.6	90 64 8.9	90 62 8.5	90 56 8.9	90 52 9.9	80 48 9.9	75 52 8.0	.. .. ..	80 53 9.2	80 44 8.9	100 41 7.2	100 38 9.0	90 43 9.1	.. .. ..
50 12 10 383	50 12 4 213	50 10 4 440	50 8 4 350	50 0 0 0	50 2 0 280	50 4 0 640	.. .. ..	50 2 2 60	50 0 0 0	50 4 2 460	50 4 0 180	50 0 0 0	.. .. ..
52 3 3 0	30 4 2 0	70 5 0 0	80 1 1 0	0 0 0 0	0 1 0 0	0 2 0 0	.. .. ..	110 0 0 1	0 0 0 0	180 0 2 0	0 2 0 0	0 0 0 0	.. .. ..
75 53 8.3	100 55 8.5	100 58 8.6	100 52 8.3	110 50 9.5	110 48 9.1	105 47 9.3	120 49 9.6	110 37 9.0	106 30 10.0	105 31 9.8	100 32 9.3	95 37 8.6	.. .. ..
50 2 0 200	50 4 2 50	50 8 4 460	50 4 0 80	50 4 0 440	50 4 0 220	50 4 2 290	50 2 2 80	50 0 0 0	50 0 0 0	50 2 2 120	50 2 0 420	50 4 0 280	.. .. ..
0 1 0 0	20 0 2 0	40 4 0 0	0 1 0 0	0 2 0 0	0 2 0 0	120 2 1 0	40 0 0 1	0 0 0 0	0 0 0 0	80 0 0 1	0 1 0 0	0 2 0 0	.. .. ..
120 10 8.6	120 13 9.4	120 13 9.5	125 13 9.7	120 15 8.4	140 15 8.9	110 11 8.3	115 15 8.5	122 12 8.8	141 11 9.8	140 9 9.3	105 11 8.9	130 11 10.7	.. .. ..
50 2 0 100	50 4 2 3,250	50 2 0 40	50 0 0 0	50 0 0 0	50 0 0 0	50 2 0 460	50 2 2 80	50 4 0 500	50 0 0 0	50 0 0 0	50 0 0 0	50 0 0 0	.. .. ..
0 1 0 0	80 2 0 0	0 1 0 0	0 0 0 0	0 0 0 0	0 0 0 0	0 1 0 0	440 0 1 0	0 2 0 0	0 0 0 0	0 0 0 0	0 0 0 0	0 0 0 0	.. .. ..
80 68 9.2	90 68 8.6	100 71 6.9	90 73 9.1	90 70 7.7	90 74 9.4	80 75 8.8	.. .. ..	85 66 7.6	90 68 9.1	105 69 8.0	90 69 8.2	84 95 7.8	.. .. ..
50 4 2 20	50 8 4 425	50 8 6 215	50 6 4 200	50 0 0 0	50 0 0 0	50 4 2 60	.. .. ..	50 8 8 115	50 6 6 73	50 6 4 867	50 14 6 337	35 72 31 2,015	.. .. ..
20 0 2 0	30 2 2 0	27 1 2 1	80 1 2 0	0 0 0 0	0 0 0 0	460 1 1 0	.. .. ..	90 0 0 4	333 0 0 3	180 1 0 2	3,320 7 0 ..	122 24 2 0	.. .. ..



TABLE

Name of village.		1939.					
		May.	June.	July.	Aug.	Sept.	Oct.
Konoka .. (Chilka Lake west).	Number of spleen examinations ..	40	40	40	40	40	40
	Spleen rate per cent ..	75	78	78	70	70	73
	Average enlarged spleen (cm. from umbilicus).	8.2	8.2	7.7	9.0	8.6	9.0
	Number of blood examinations ..	40	40	40	40	40	40
	Parasite rate per cent ..	5	8	8	8	10	5
	Gametocyte rate per cent ..	2	5	3	3	5	0
	Average positive parasite count per c.mm. of blood.	50	33	207	40	135	120
	Species of parasite	Asexual ..					
		Sexual ..					
		<i>P. falciparum</i> ..					
		<i>P. vivax</i> ..					
Sabilia (Rambha) .. (Chilka Lake west).	Number of spleen examinations ..	80	80	100	100	100	100
	Spleen rate per cent ..	49	50	48	42	36	35
	Average enlarged spleen (cm. from umbilicus).	9.5	8.2	9.1	9.3	8.6	9.5
	Number of blood examinations ..	80	50	50	50	50	50
	Parasite rate per cent ..	31	24	14	8	12	10
	Gametocyte rate per cent ..	20	18	10	8	10	8
	Average positive parasite count per c.mm. of blood.	1,665	642	423	25	248	400
	Species of parasite	Asexual ..					
		Sexual ..					
		<i>P. falciparum</i> ..					
		<i>P. vivax</i> ..					
Bahadrapalli .. (Chilka Lake west).	Number of spleen examinations ..	..	40	35	40	36	40
	Spleen rate per cent ..	..	40	46	35	28	28
	Average enlarged spleen (cm. from umbilicus).	..	10.6	9.4	10.6	9.8	10.0
Gandapalli .. (Chilka Lake west).	Number of spleen examinations ..	35	40	40	40	31	30
	Spleen rate per cent ..	20	33	25	20	26	23
	Average enlarged spleen (cm. from umbilicus).	7.8	8.5	9.0	9.0	10.0	10.0
	Number of blood examinations ..	35	40	40	40	31	30
	Parasite rate per cent ..	15	8	3	5	10	10
	Gametocyte rate per cent ..	11	5	3	5	3	3
	Average positive parasite count per c.mm. of blood.	770	106	20	3,025	423	417
	Species of parasite	Asexual ..					
		Sexual ..					
		<i>P. falciparum</i> ..					
		<i>P. vivax</i> ..					
Garh Humma ..	Number of spleen examinations ..	..	40	33	40	40	45
	Spleen rate per cent ..	..	70	67	48	48	44
	Average enlarged spleen (cm. from umbilicus).	..	8.0	8.5	8.6	8.8	8.3



## IX—contd.

1940.

Nov.	Dec.	Jan.	Feb.	March.	April.	May.	June.	July.	Aug.	Sept.	Oct.	Nov.	Dec.
36 75 8.1	.. .. ..	40 75 8.6	45 78 8.9	.. .. ..	57 61 8.3	45 69 8.1	.. .. ..	41 66 9.0	42 67 8.5	40 55 9.5	40 65 9.6	40 68 9.1	.. .. ..
36 6 3 7,550	.. .. .. ..	40 5 0 210	45 2 2 200	.. .. .. ..	50 4 0 60	45 9 9 50	.. .. .. ..	40 8 5 127	42 7 7 460	40 0 3 0	40 3 3 460	40 10 5 700	.. .. .. ..
500 2 0 0	.. .. .. ..	0 2 0 0	60 0 1 0	.. .. .. ..	0 2 0 0	155 3 0 1	.. .. .. ..	100 2 0 1	5,067 0 0 3	0 0 0 0	200 0 0 1	60 2 1 1	.. .. .. ..
100 37 8.5	100 38 8.5	100 35 11.5	100 36 9.1	100 33 8.7	100 41 8.2	100 55 8.2	100 60 7.9	95 64 8.0	100 56 8.7	100 47 9.3	100 46 9.1	100 61 8.0	.. .. ..
50 6 2 767	50 6 4 347	50 4 2 112	50 2 2 800	50 2 0 80	50 14 10 1,925	50 20 6 112	50 10 8 476	50 10 6 3,776	50 14 12 637	50 4 4 340	50 2 0 120	50 20 6 3,312	50 32 12 5,733
50 2 2 0	75 0 3 0	10 1 1 0	200 0 1 0	0 1 0 0	872 7 0 0	487 10 0 0	410 4 2 0	340 4 0 1	463 2 1 4	1,200 1 1 0	0 1 0 0	1,887 8 1 1	507 14 3 0
35 26 9.6	35 20 10.7	35 23 9.6	40 25 10.4	40 23 10.3	32 22 10.9	32 25 9.4	46 17 9.1	42 14 10.7	35 17 10.3	30 17 10.2	30 20 10.5	37 57 8.1	.. .. ..
30 23 10.6	35 22 10.1	30 23 9.3	31 22 9.4	30 20 9.5	30 23 9.7	30 27 9.8	.. .. ..	32 16 9.4	.. .. ..	.. .. ..	.. .. ..	.. .. ..	.. .. ..
30 0 0 0	30 2 2 80	30 0 0 0	32 6 0 120	30 0 0 0	30 3 0 80	30 3 3 40	.. .. .. ..	30 0 0 0	.. .. .. ..	.. .. .. ..	.. .. .. ..	.. .. .. ..	.. .. .. ..
0 0 0 0	40 1 0 0	0 0 0 0	0 0 2 0	0 0 0 0	0 0 0 0	240 1 0 0	.. .. .. ..	0 0 0 0	.. .. .. ..	.. .. .. ..	.. .. .. ..	.. .. .. ..	.. .. .. ..
37 46 7.6	40 48 8.3	40 43 6.8	40 43 8.5	45 47 7.3	42 48 8.3	40 68 7.7	47 51 7.6	40 53 8.4	36 50 7.7	40 50 8.4	40 48 8.1	40 50 8.6	.. .. ..



## TABLE

Name of village.		1939.							
		May.	June.	July.	Aug.	Sept.	Oct.		
Tolo Humma ..	{	Number of spleen examinations ..	70	110	110	110	100	110	
		Spleen rate per cent ..	50	36	36	31	28	31	
		Average enlarged spleen (cm. from umbilicus).	9.3	8.6	8.7	8.1	8.1	9.2	
		Number of blood examinations ..	37	50	50	50	50	50	
		Parasite rate per cent ..	7	10	6	14	8	2	
		Gametocyte rate per cent ..	0	10	6	8	6	2	
		Average positive parasite count per c.mm. of blood.	75	225	37	231	105	1,200	
		Species of parasite	Asexual ..	0	192	27	35	40	100
			<i>P. falciparum</i> ..	1	2	1	4	2	0
			<i>P. vivax</i> ..	1	4	2	3	2	1
<i>P. malaria</i> ..	0		0	0	0	0	0		
Humma Salt Lines ..	{	Number of spleen examinations ..	43	35	33	30	30	34	
		Spleen rate per cent ..	51	49	39	27	23	24	
		Average enlarged spleen (cm. from umbilicus).	8.8	8.9	8.0	9.1	9.0	9.0	
		Number of blood examinations ..	43	35	33	30	30	34	
		Parasite rate per cent ..	19	8	3	10	7	9	
		Gametocyte rate per cent ..	15	6	3	10	7	3	
		Average positive parasite count per c.mm. of blood.	900	108	200	367	60	157	
		Species of parasite	Asexual ..	92	50	50	90	40	100
			<i>P. falciparum</i> ..	6	0	0	0	0	0
			<i>P. vivax</i> ..	2	3	1	3	2	3
<i>P. malaria</i> ..	0		0	0	0	0	0		
Dubrakudi (Chilka Lake south).	{	Number of spleen examinations ..	55	56	60	55	55	55	
		Spleen rate per cent ..	89	79	80	72	60	62	
		Average enlarged spleen (cm. from umbilicus).	7.7	7.7	7.7	7.6	8.1	8.7	
		Number of blood examinations ..	55	56	50	50	50	50	
		Parasite rate per cent ..	18	7	8	8	8	2	
		Gametocyte rate per cent ..	15	7	2	6	6	2	
		Average positive parasite count per c.mm. of blood.	830	106	73	225	225	100	
		Species of parasite	Asexual ..	169	58	20	60	87	200
			<i>P. falciparum</i> ..	6	1	2	2	1	0
			<i>P. vivax</i> ..	4	1	2	2	3	1
<i>P. malaria</i> ..	1		2	0	0	0	0		
Lakhanapur (Chilka Lake south).	{	Number of spleen examinations ..	..	..	..	..	..	..	
		Spleen rate per cent ..	..	..	..	..	..	..	
		Average enlarged spleen (cm. from umbilicus).	..	..	..	..	..	..	
			..	..	..	..	..	..	
Proyagi (Chilka Lake south).	{	Number of spleen examinations ..	..	60	..	..	..	70	
		Spleen rate per cent ..	..	93	..	..	..	..	90
		Average enlarged spleen (cm. from umbilicus).	..	6.7	..	..	..	..	7.1
			..	..	..	..	..	..	



IX—*contd.*

1940.

Nov.	Dec.	Jan.	Feb.	March.	April.	May.	June.	July.	Aug.	Sept.	Oct.	Nov.	Dec.
100 31 8·7	70 31 8·2	100 28 8·4	100 29 8·9	110 27 8·8	110 24 8·2	100 22 8·0	120 28 8·9	106 23 8·7	110 28 9·1	125 28 9·1	110 28 8·5	102 27 9·3	.. .. ..
50 6 4 673	50 4 2 50	50 4 0 30	50 0 0 0	50 2 2 40	50 0 0 0	50 2 0 80	50 4 0 250	50 4 0 390	50 4 2 390	50 4 2 8,400	50 4 0 8,360	50 8 6 1,740	.. .. .. ..
200 1 2 0	20 0 2 0	0 2 0 0	0 0 0 0	20 0 0 1	0 0 0 0	0 1 0 0	0 0 2 0	0 2 0 0	500 2 0 0	260 2 0 0	0 2 0 0	400 3 1 0	.. .. .. ..
30 23 8·9	30 23 9·1	30 30 9·1	35 23 6·4	30 27 8·1	30 27 8·4	30 30 7·7	30 36 7·5	35 29 8·1	33 36 9·2	31 36 7·3	30 37 8·1	30 37 8·1	.. .. .. ..
30 3 3 1,200	30 0 0 0	30 3 3 60	35 2 2 200	30 0 0 0	30 0 0 0	30 3 3 80	30 13 7 720	35 9 6 260	33 9 6 373	30 7 3 750	30 10 0 1,120	30 13 3 640	.. .. .. ..
200 0 1 0	0 0 0 0	20 1 0 0	40 0 1 0	0 0 0 0	0 0 0 0	800 1 0 0	130 3 1 0	100 1 1 1	440 2 1 0	880 2 0 0	0 3 0 0	20 4 0 0	.. .. .. ..
55 83 8·4	55 75 8·8	60 70 9·5	60 70 8·7	55 73 8·6	56 79 7·7	55 80 8·3	60 82 8·0	60 77 7·8	55 76 8·6	60 73 7·8	55 76 7·7	60 88 7·8	.. .. .. ..
50 6 2 430	50 12 8 597	50 6 6 253	50 8 4 3,235	50 4 4 1,170	50 6 2 3,065	50 20 16 270	50 12 12 150	50 14 10 331	50 10 10 220	50 16 14 318	50 12 4 483	50 18 2 1,252	.. .. .. ..
50 1 2 0	80 3 3 0	40 2 0 1	250 1 3 0	40 1 0 0	80 2 0 0	460 5 0 5	253 1 0 5	220 3 1 3	280 0 0 5	229 2 0 6	310 3 0 3	530 6 0 3	.. .. .. ..
.. .. ..	.. .. ..	.. .. ..	.. .. ..	47 68 9·1	42 69 8·1	50 72 7·7	42 74 8·7	44 73 7·7	40 60 7·3	46 59 7·4	55 62 8·2	50 68 7·7	.. .. ..
70 91 7·2	70 92 7·3	70 93 6·8	65 92 7·5	72 92 7·7	70 94 6·8	70 85 7·1	65 91 8·9	60 92 7·9	66 92 7·5	65 85 7·3	60 90 7·5	65 100 7·2	.. .. ..



TABLE

Name of village.		1939.								
		May.	June.	July.	Aug.	Sept.	Oct.			
Padapodoro (near Chatrapur).	{	Number of spleen examinations	..	..	..	80	80	80		
		Spleen rate per cent	..	..	..	26	29	34		
		Average enlarged spleen (cm. from umbilicus)	..	..	..	10.0	9.3	9.6		
Hummiri .. (near Chatrapur).	{	Number of spleen examinations	..	..	..	85	80	80		
		Spleen rate per cent	..	..	..	8	11	8		
		Average enlarged spleen (cm. from umbilicus).	..	..	..	10.3	9.9	10.7		
Komarobegopalli (near Chatrapur).	{	Number of spleen examinations	..	..	..	100	80	80		
		Spleen rate per cent	..	..	..	8	6	6		
		Average enlarged spleen (cm. from umbilicus).	..	..	..	9.4	9.8	11.0		
Chatrapur (Reserve Police Lines).	{	Number of spleen examinations	..	75	75	72	80	90	70	
		Spleen rate per cent	..	32	32	26	16	14	16	
		Average enlarged spleen (cm. from umbilicus).	..	8.9	9.2	9.5	9.6	8.5	8.7	
		Number of blood examinations	..	75	50	50	50	50	50	
		Parasite rate per cent	..	6	6	6	0	4	0	
		Gametoocyte rate per cent	..	3	6	4	0	4	0	
		Average positive parasite count per c.mm. of blood.	{	775	1,933	1,173	0	140	0	
			Asexual	..						
			Sexual	..	75	333	83	0	110	0
			<i>P. falciparum</i>	..	2	1	2	0	0	0
			<i>P. vivax</i>	..	2	2	0	2	0	0
			<i>P. malarie</i>	..	1	0	0	0	0	0
			Species of parasite							
Chatrapur (Reddika Street).	{	Number of spleen examinations	..	40	85	85	85	80	80	
		Spleen rate per cent	..	8	5	5	2	4	3	
		Average enlarged spleen (cm. from umbilicus).	..	12.3	9.7	10.3	11.0	10.1	9.0	
		Number of blood examinations	..	40	50	50	50	50	50	
		Parasite rate per cent	..	0	0	0	0	0	0	
		Gametoocyte rate per cent	..	0	0	0	0	0	0	
		Average positive parasite count per c.mm. of blood.	{	0	0	0	0	0	0	
			Asexual	..						
			Sexual	..	0	0	0	0	0	0
			<i>P. falciparum</i>	..	0	0	0	0	0	0
			<i>P. vivax</i>	..	0	0	0	0	0	0
			<i>P. malarie</i>	..	0	0	0	0	0	0
			Species of parasite							
Chatrapur (Agarharam Street).	{	Number of spleen examinations	..	..	..	..	..	..		
		Spleen rate per cent	..	..	..	..	..	..		
		Average enlarged spleen (cm. from umbilicus).	..	..	..	..	..	..		
Chatrapur (Tampara huts).	{	Number of spleen examinations	..	..	..	..	..	..		
		Spleen rate per cent	..	..	..	..	..	..		
		Average enlarged spleen (cm. from umbilicus).	..	..	..	..	..	..		



IX—contd.

1940.													
Nov.	Dec.	Jan.	Feb.	Mar.	April.	May.	June.	July.	Aug.	Sept.	Oct.	Nov.	Dec.
80 31 8.8	90 33 9.4	90 29 9.0	90 30 9.5	90 29 9.0	90 24 8.3	80 30 9.2	.. .. ..	80 31 8.6	100 28 9.4	85 22 9.7	100 23 7.9	110 30 8.5	.. .. ..
80 9 10.1	80 10 9.4	80 10 10.1	80 13 8.9	80 8 9.2	80 4 9.0	80 11 8.7	.. .. ..	75 41 7.8	80 41 8.4	90 40 8.5	80 40 8.3	85 37 8.8	.. .. ..
90 7 9.2	80 8 8.3	80 10 9.6	80 9 9.3	85 8 10.4	80 5 7.2	86 7 9.2	.. .. ..	68 17 8.3	75 15 8.8	80 13 8.4	80 10 8.3	80 11 8.8	.. .. ..
75 16 9.1	80 19 9.7	80 20 9.3	90 21 9.1	80 21 8.8	80 19 9.4	80 14 9.5	.. .. ..	75 19 8.4	75 20 8.6	80 15 8.7	80 16 8.1	75 19 8.6	.. .. ..
50 4 2 450	50 0 0 0	50 6 4 650	50 0 0 0	50 0 0 0	50 0 0 0	50 2 0 80	.. .. .. ..	50 4 0 700	50 0 0 0	50 4 2 320	50 4 0 440	50 6 4 793	.. .. .. ..
20 1 1 0	0 0 0 0	40 2 1 0	0 0 0 0	0 0 0 0	0 2 0 0	0 1 0 0	.. .. .. ..	0 2 0 0	0 .. 0 0	180 2 0 0	0 2 0 0	690 2 1 0	.. .. .. ..
90 4 9.5	80 4 11.0	80 3 10.5	85 1 9.2	80 3 10.0	80 1 9.0	80 1 11.0	.. .. ..	80 3 10.0	90 1 13.0	85 2 13.0	95 3 10.3	100 1 10.0	.. .. ..
50 2 0 20	50 0 0 0	50 2 0 40	50 0 0 0	50 0 0 0	50 0 0 0	50 0 0 0	.. .. .. ..	50 0 0 0	50 0 0 0	50 0 0 0	50 0 0 0	50 0 0 0	.. .. .. ..
0 0 1 0	0 0 0 0	0 1 0 0	0 0 0 0	0 0 0 0	0 0 0 0	0 0 0 0	.. .. .. ..	0 0 0 0	0 0 0 0	0 0 0 0	0 0 0 0	0 0 0 0	.. .. .. ..
.. .. ..	34 9 8.0	35 11 8.8	35 11 8.8	35 11 9.3	35 11 9.5	35 26 10.2	.. .. ..	.. .. ..	35 23 9.3	.. .. ..	.. .. ..	.. .. ..	.. .. ..
.. .. ..	.. .. ..	13 92 10.0	9 78 10.1	12 83 9.6	12 75 10.4	12 67 10.6	.. .. ..	10 60 8.2	9 68 9.8	8 50 10.8	8 50 10.8	10 60 10.0	.. .. ..



## TABLE

Name of village.		1939.							
		May.	June.	July.	Aug.	Sept.	Oct.		
Haripur-Bandra (Gopalpur Creek).	{	Number of spleen examinations ..	..	..	55	..	..	80	
		Spleen rate per cent ..	..	..	93	..	..	79	
		Average enlarged spleen (cm. from umbilicus).	..	..	10.2	..	..	7.8	
		Number of blood examinations ..	..	..	50	..	..	50	
		Parasite rate per cent ..	..	..	20	..	..	30	
		Gametocyte rate per cent ..	..	..	16	..	..	20	
		Average positive parasite count per c.mm. of blood.	{ Asexual ..	..	..	800	..	..	750
			{ Sexual ..	..	..	125	..	..	173
			{ <i>P. falciparum</i> ..	..	..	3	..	..	8
			{ <i>P. vivax</i> ..	..	..	9	..	..	8
	{ <i>P. malariae</i> ..	..	..	0	..	..	0		
Gopalpur ..	{	Number of spleen examinations ..	..	..	90	..	..	80	
		Spleen rate per cent ..	..	..	29	..	..	21	
		Average enlarged spleen (cm. from umbilicus).	..	..	10.2	..	..	9.2	
		Number of blood examinations ..	..	..	50	..	..	50	
		Parasite rate per cent ..	..	..	0	..	..	4	
		Gametocyte rate per cent ..	..	..	0	..	..	2	
		Average positive parasite count per c.mm. of blood.	{ Asexual ..	..	..	0	..	..	120
			{ Sexual ..	..	..	0	..	..	100
			{ <i>P. falciparum</i> ..	..	..	0	..	..	1
			{ <i>P. vivax</i> ..	..	..	0	..	..	1
	{ <i>P. malariae</i> ..	..	..	0	..	..	0		



## IX—contd.

1940.													
Nov.	Dec.	Jan.	Feb.	Mar.	April.	May.	June.	July.	Aug.	Sept.	Oct.	Nov.	Dec.
90	70	52	51	50	51	..	40	52	51	50	42	..	..
81	83	85	84	86	86	..	83	90	92	90	95	..	..
8.1	7.1	7.1	6.9	8.1	6.5	..	7.6	8.3	7.9	7.1	6.3	..	..
50	50	50	50	50	50	..	40	50	50	50	42	..	..
16	8	14	6	6	8	..	10	14	12	16	33	..	..
10	6	12	4	2	4	..	8	6	6	2	10	..	..
355	8,430	634	310	200	140	..	195	1,366	163	2,203	1,124	..	..
72	33	263	90	67	130	..	93	133	160	120	665	..	..
4	2	4	0	1	2	..	0	4	2	7	11	..	..
4	3	3	0	1	0	..	0	0	0	0	0	..	..
0	0	1	3	1	2	..	4	3	4	0	3	..	..
90	90	80	100	100	100	..	100	105	105	95	100	..	..
17	19	20	17	23	18	..	15	17	27	21	26	..	..
7.6	9.1	9.1	9.7	9.5	9.3	..	10.3	9.6	9.4	9.1	8.7	..	..
50	50	50	50	50	50	..	50	50	50	50	50	..	..
4	4	6	0	0	0	..	2	6	8	14	14	..	..
2	0	4	0	0	0	..	0	4	2	8	4	..	..
40	60	373	0	0	0	..	800	2,520	385	440	1,163	..	..
20	0	120	0	0	0	..	0	150	360	1,000	100	..	..
0	1	0	0	0	0	..	0	3	3	7	7	..	..
2	1	3	0	0	0	..	0	0	1	0	0	..	..
0	0	0	0	0	0	..	0	0	0	0	0	..	..



TABLE

Name of village.		Jan.	Feb.	Mar.	April.
Soran .. (Chilka Lake west).	Number of spleen examinations ..	100	86	80	90
	Spleen rate per cent ..	45	48	46	51
	Average enlarged spleen (cm. from umbilicus).	9.7	9.1	9.7	9.9
	Number of blood examinations ..	50	50	50	50
	Parasite rate per cent ..	2	2	4	2
	Gametocyte rate per cent ..	2	0	0	0
	Average positive parasite count per c.mm. of blood.	120	320	960	3,200
	Species of parasite	Asexual ..			
		Sexual ..			
		<i>P. falciparum</i> ..			
		<i>P. vivax</i> ..			
Sana Nairi .. (Chilka Lake west).	Number of spleen examinations ..	110	110	102	111
	Spleen rate per cent ..	33	43	48	70
	Average enlarged spleen (cm. from umbilicus).	9.1	8.8	9.5	8.7
	Number of blood examinations ..	50	50	50	50
	Parasite rate per cent ..	10	6	8	18
	Gametocyte rate per cent ..	4	0	2	4
	Average positive parasite count per c.mm. of blood.	948	207	420	844
	Species of parasite	Asexual ..			
		Sexual ..			
		<i>P. falciparum</i> ..			
		<i>P. vivax</i> ..			
Balugaon .. (Chilka Lake west).	Number of spleen examinations ..	125	140	118	140
	Spleen rate per cent ..	11	11	10	12
	Average enlarged spleen (cm. from umbilicus).	9.4	9.1	10.0	9.6
	Number of blood examinations ..	50	50	50	50
	Parasite rate per cent ..	0	0	0	0
	Gametocyte rate per cent ..	0	0	0	0
	Average positive parasite count per c.mm. of blood.	0	0	0	0
	Species of parasite	Asexual ..			
		Sexual ..			
		<i>P. falciparum</i> ..			
		<i>P. vivax</i> ..			
Kesopur .. (Chilka Lake west).	Number of spleen examinations ..	90	95	100	85
	Spleen rate per cent ..	100	100	100	99
	Average enlarged spleen (cm. from umbilicus).	7.0	7.8	7.7	7.1
	Number of blood examinations ..	40	34	34	29
	Parasite rate per cent ..	25	50	27	14
	Gametocyte rate per cent ..	10	9	6	7
	Average positive parasite count per c.mm. of blood.	1,048	640	653	3,255
	Species of parasite	Asexual ..			
		Sexual ..			
		<i>P. falciparum</i> ..			
		<i>P. vivax</i> ..			



IX—contd.

1941.								1942.		
May.	June.	July.	Aug.	Sept.	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.
..	85	..	97	..	80	..	75	..	..	..
..	44	..	39	..	38	..	80	..	..	..
..	9.5	..	9.3	..	10.4	..	8.4	..	..	..
..	50	..	50	..	50	..	14	..	..	..
..	2	..	0	..	6	..	79	..	..	..
..	0	..	0	..	4	..	14	..	..	..
..	1,820	..	0	..	947	..	1,369	..	..	..
..	0	..	0	..	70	..	660	..	..	..
..	1	..	0	..	2	..	11	..	..	..
..	0	..	0	..	1	..	0	..	..	..
..	0	..	0	..	0	..	0	..	..	..
95	110	115	102	81	105	120	..	..	120	..
75	76	79	76	77	75	83	..	..	92	..
8.8	8.6	8.7	9.7	9.1	8.5	9.1	..	..	8.2	..
50	50	50	50	50	50	50	..	..	50	..
22	18	2	4	14	12	16	..	..	36	..
6	2	0	2	6	6	2	..	..	8	..
596	680	480	1,080	800	557	1,700	..	..	1,661	..
340	20	0	220	93	120	60	..	..	185	..
11	8	1	1	4	3	8	..	..	15	..
0	0	0	0	0	3	0	..	..	1	..
0	1	0	1	3	0	0	..	..	2	..
125	107	120	130	115	150	143	110	..	..	120
12	11	10	8	10	11	8	15	..	..	12
9.1	9.0	7.7	9.4	9.8	9.9	9.8	9.2	..	..	7.3
50	50	50	50	50	50	50	50	..	..	..
0	0	2	0	0	0	0	0	..	..	..
0	0	0	0	0	0	0	0	..	..	..
0	0	840	0	0	0	0	0	..	..	..
0	0	0	0	0	0	0	0	..	..	..
0	0	1	0	0	0	0	0	..	..	..
0	0	0	0	0	0	0	0	..	..	..
0	0	0	0	0	0	0	0	..	..	..
90	105	94	93	..	..	112	..	115	..	112
99	98	99	99	..	..	97	..	98	..	98
7.7	8.6	8.9	8.6	..	..	9.2	..	8.5	..	8.1
40	48	40	28	..	..	33	..	35	..	35
10	10	13	14	..	..	36	..	23	..	29
0	2	5	7	..	..	15	..	11	..	11
380	632	1,236	720	..	..	1,758	..	1,543	..	406
0	20	100	290	..	..	1,680	..	395	..	275
4	5	5	2	..	..	9	..	7	..	7
0	0	0	0	..	..	3	..	0	..	0
0	0	0	2	..	..	2	..	1	..	3



### TABLE

Name of village.		Jan.	Feb.	Mar.	April.
Kallikota .. (Chilka Lake west).	Number of spleen examinations ..	125	115	125	135
	Spleen rate per cent ..	38	39	39	39
	Average enlarged spleen (cm. from umbilicus).	9.0	8.6	8.6	8.5
	Number of blood examinations ..	50	50	50	50
	Parasite rate per cent ..	14	12	10	6
	Gametocyte rate per cent ..	6	2	0	0
	Average positive parasite count per c.mm. of blood.	554	897	536	433
	Species of parasite { Asexual ..	147	320	0	0
	{ Sexual ..	7	6	5	2
	{ <i>P. falciparum</i> ..	1	0	0	1
Konoka .. (Chilka Lake west).	Number of spleen examinations ..	..	..	..	40
	Spleen rate per cent ..	..	..	..	80
	Average enlarged spleen (cm. from umbilicus).	..	..	..	8.7
	Number of blood examinations ..	..	..	..	40
	Parasite rate per cent ..	..	..	..	7.5
	Gametocyte rate per cent ..	..	..	..	0
	Average positive parasite count per c.mm. of blood.	..	..	..	1,393
	Species of parasite { Asexual ..	..	..	..	0
	{ Sexual ..	..	..	..	3
	{ <i>P. falciparum</i> ..	..	..	..	0
Sabilia (Rambha) .. (Chilka Lake west).	Number of spleen examinations ..	100	100	105	105
	Spleen rate per cent ..	80	82	77	76
	Average enlarged spleen (cm. from umbilicus).	7.9	7.1	8.1	8.6
	Number of blood examinations ..	50	50	50	50
	Parasite rate per cent ..	22	24	10	8
	Gametocyte rate per cent ..	4	8	2	4
	Average positive parasite count per c.mm. of blood.	767	833	1,300	215
	Species of parasite { Asexual ..	1,310	110	160	120
	{ Sexual ..	7	10	5	4
	{ <i>P. falciparum</i> ..	5	1	0	0
Bahadrapalli .. (Chilka Lake west).	Number of spleen examinations ..	45	45	38	42
	Spleen rate per cent ..	80	78	76	69
	Average enlarged spleen (cm. from umbilicus).	9.8	10.5	10.4	10.1
Rambha .. (Chilka Lake west).	Number of spleen examinations ..	..	155	..	..
	Spleen rate per cent ..	..	52	..	..
	Average enlarged spleen (cm. from umbilicus).	..	8.3	..	..



IX—contd.

1941.								1942.		
May.	June.	July.	Aug.	Sept.	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.
90 40 8.3	115 40 9.4	132 39 9.0	.. .. ..	.. .. ..	.. .. ..	115 20 9.0	.. .. ..	.. .. ..	.. .. ..	120 39 8.5
50 8 4 7.50	50 6 2 3.93	50 2 0 8.40	.. .. .. ..	.. .. .. ..	.. .. .. ..	50 0 0 0	.. .. .. ..	.. .. .. ..	.. .. .. ..	.. .. .. ..
890 4 0 0	440 3 1 0	0 1 0 0	.. .. .. ..	.. .. .. ..	.. .. .. ..	0 0 0 0	.. .. .. ..	.. .. .. ..	.. .. .. ..	.. .. .. ..
.. .. ..	.. .. ..	.. .. ..	.. .. ..	.. .. ..	.. .. ..	42 83 9.5	.. .. ..	43 81 10.2	.. .. ..	45 80 9.8
.. .. ..	.. .. ..	.. .. ..	.. .. ..	.. .. ..	.. .. ..	.. .. ..	.. .. ..	.. .. ..	.. .. ..	.. .. ..
.. .. ..	.. .. ..	.. .. ..	.. .. ..	.. .. ..	.. .. ..	.. .. ..	.. .. ..	.. .. ..	.. .. ..	.. .. ..
95 79 7.8	104 78 8.1	120 70 8.0	105 72 9.2	125 68 8.9	95 62 9.7	95 60 9.3	112 63 9.9	110 66 9.3	.. .. ..	105 60 9.1
50 2 0 1,820	50 6 2 3.63	50 4 0 7.00	50 2 2 8.0	50 4 4 2.20	50 4 2 1,160	50 6 2 260	50 2 0 400	50 8 0 305	.. .. .. ..	50 4 2 230
0 1 0 0	40 3 0 0	0 2 0 0	120 0 0 1	210 0 1 1	100 2 1 0	60 1 1 1	0 1 0 0	0 4 0 0	.. .. .. ..	560 1 0 1
47 08 10.4	35 57 9.9	38 53 10.3	51 51 11.3	30 40 10.6	25 38 11.2	.. .. ..	.. .. ..	35 31 11.2	.. .. ..	41 29 10.8
.. .. ..	.. .. ..	.. .. ..	150 47 8.8	.. .. ..	.. .. ..	150 37 9.3	.. .. ..	180 30 9.6	.. .. ..	164 27 9.4



TABLE

Name of village.		Jan.	Feb.	Mar.	April.
Garh Humma ..	Number of spleen examinations ..	35	45	40	36
	Spleen rate per cent ..	80	80	80	81
	Average enlarged spleen (om. from umbilicus).	8.2	7.1	7.5	7.8
Tolo Humma ..	Number of spleen examinations ..	95	105	105	125
	Spleen rate per cent ..	40	37	37	35
	Average enlarged spleen (om. from umbilicus).	8.7	8.8	8.5	9.0
	Number of blood examinations ..	50	50	50	50
	Parasite rate per cent ..	16	2	4	8
	Gametocyte rate per cent ..	4	0	0	2
	Average positive parasite count per c.mm. of blood.	933	220	420	780
	Species of parasite {	Asexual ..	Sexual ..	P. falciparum ..	P. vivax ..
	Species of parasite {	P. falciparum ..	P. vivax ..	P. malarie ..	
Humma Salt Lines ..	Number of spleen examinations ..	34	30	29	34
	Spleen rate per cent ..	53	50	52	53
	Average enlarged spleen (om. from umbilicus).	8.6	8.5	8.1	8.0
	Number of blood examinations ..	30	30	29	30
	Parasite rate per cent ..	30	13	10	10
	Gametocyte rate per cent ..	10	3	7	3
	Average positive parasite count per c.mm. of blood.	5,053	1,650	1,053	880
	Species of parasite {	Sexual ..	P. falciparum ..	P. vivax ..	P. malarie ..
Dubrakudi (Chilka Lake south).	Number of spleen examinations ..	55	52	60	52
	Spleen rate per cent ..	91	92	95	92
	Average enlarged spleen (om. from umbilicus).	8.2	6.6	7.0	7.5
	Number of blood examinations ..	50	50	50	50
	Parasite rate per cent ..	34	22	12	12
	Gametocyte rate per cent ..	8	6	2	0
	Average positive parasite count per c.mm. of blood.	832	1,467	587	183
	Species of parasite {	Sexual ..	P. falciparum ..	P. vivax ..	P. malarie ..
Lakhanapur (Chilka Lake south).	Number of spleen examinations ..	40	45	50	50
	Spleen rate per cent ..	70	73	82	82
	Average enlarged spleen (om. from umbilicus).	7.3	6.8	6.9	7.7



IX—contd.

1941.								1942.		
May.	June.	July.	Aug.	Sept.	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.
40 75 7.6	43 74 8.1	38 71 8.4	38 66 8.1	.. .. ..	42 50 8.0	.. .. ..	38 53 8.7	.. .. ..	.. .. ..	45 53 8.3
100 35 8.5	113 35 8.7	96 30 9.5	105 25 9.6	.. .. ..	112 29 9.0	.. .. ..	88 30 9.5	.. .. ..	.. .. ..	120 24 8.5
50 4 0 100	50 0 0 0	50 0 0 0	50 0 0 0	.. .. .. ..	50 0 0 0	.. .. .. ..	50 2 0 100	.. .. .. ..	.. .. .. ..	50 4 2 470
0 2 0 0	0 0 0 0	0 0 0 0	0 0 0 0	.. .. .. ..	0 0 0 0	.. .. .. ..	0 1 0 0	.. .. .. ..	.. .. .. ..	80 2 0 0
35 40 9.1	25 40 9.5	35 26 7.6	32 25 9.4	.. .. ..	36 22 8.9	.. .. ..	40 30 8.6	.. .. ..	.. .. ..	36 33 9.5
34 3 3 100	25 0 0 0	31 7 3 1,950	32 6 6 2,760	.. .. .. ..	35 3 0 4,600	.. .. .. ..	30 3 0 2,400	.. .. .. ..	.. .. .. ..	.. .. .. ..
480 1 0 0	0 0 0 0	180 1 0 1	570 1 1 0	.. .. .. ..	0 1 0 0	.. .. .. ..	0 1 0 0	.. .. .. ..	.. .. .. ..	.. .. .. ..
55 95 7.4	67 94 8.4	60 92 9.1	60 90 8.9	60 92 8.3	60 87 9.2	52 94 9.3	55 89 9.1	.. .. ..	.. .. ..	55 93 7.8
50 22 4 225	50 18 8 617	50 12 4 793	50 16 10 1,223	50 12 12 180	50 10 4 352	50 .. .. ..	50 18 2 838	.. .. .. ..	.. .. .. ..	50 24 10 552
170 9 0 2	135 7 1 1	260 4 0 2	552 4 1 4	377 0 0 6	70 3 0 2	.. .. .. ..	120 8 1 0	.. .. .. ..	.. .. .. ..	152 7 0 5
55 89 8.7	41 90 7.8	50 86 9.1	45 89 8.9	48 98 9.0	41 90 10.1	48 87 9.0	30 83 9.8	.. .. ..	.. .. ..	42 98 8.6



TABLE

Name of village.		Jan.	Feb.	Mar.	April.
Proyagi .. (Chilka Lake south).	Number of spleen examinations ..	61	64	65	71
	Spleen rate per cent ..	100	98	100	100
	Average enlarged spleen (cm. from umbilicus).	7.5	7.7	6.8	8.1
Padapodoro .. (near Chatrapur).	Number of spleen examinations ..	95	95	80	80
	Spleen rate per cent ..	33	28	34	34
	Average enlarged spleen (cm. from umbilicus).	8.7	9.6	9.0	8.7
Hummiri .. (near Chatrapur).	Number of spleen examinations ..	80	95	90	160
	Spleen rate per cent ..	40	43	41	41
	Average enlarged spleen (cm. from umbilicus).	8.4	8.6	9.8	9.0
Komarobegopalli .. (near Chatrapur).	Number of spleen examinations ..	80	82	85	100
	Spleen rate per cent ..	15	15	16	14
	Average enlarged spleen (cm. from umbilicus).	8.6	8.5	8.9	9.5
Chatrapur .. (Reserve Police Lines).	Number of spleen examinations ..	85	75	70	70
	Spleen rate per cent ..	20	23	34	27
	Average enlarged spleen (cm. from umbilicus).	8.9	8.2	9.0	8.9
	Number of blood examinations ..	50	50	50	50
	Parasite rate per cent ..	8	6	10	6
	Gametocyte rate per cent ..	8	0	0	4
	Average positive parasite count per c.mm. of blood.	1,175	420	1,016	530
	Sexual ..	110	0	0	340
	<i>P. falciparum</i> ..	3	3	5	3
	<i>P. vivax</i> ..	2	0	0	0
	<i>P. malariae</i> ..	0	0	0	0
Chatrapur .. (Reddika Street).	Number of spleen examinations ..	80	74	80	90
	Spleen rate per cent ..	0	1	3	2
	Average enlarged spleen (cm. from umbilicus).	..	8.0	9.0	9.0
	Number of blood examinations ..	50	50	50	50
	Parasite rate per cent ..	0	0	0	0
	Gametocyte rate per cent ..	0	0	0	0
	Average positive parasite count per c.mm. of blood.	0	0	0	0
	Sexual ..	0	0	0	0
	<i>P. falciparum</i> ..	0	0	0	0
	<i>P. vivax</i> ..	0	0	0	0
	<i>P. malariae</i> ..	0	0	0	0
Chatrapur .. (Bikkapalli).	Number of spleen examinations ..	50	65	65	70
	Spleen rate per cent ..	10	12	12	13
	Average enlarged spleen (cm. from umbilicus).	9.8	8.6	9.4	8.4



IX—contd.

1941.								1942.		
May.	June.	July.	Aug.	Sept.	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.
65 99 7.7	60 97 8.1	58 97 9.5	65 99 8.9	68 97 10.7	65 99 9.2	64 97 8.8	45 98 9.0	61 100 8.5	.. .. ..	60 100 7.7
90 29 9.8	100 21 8.9	95 26 9.8	105 26 10.1	95 26 10.4	.. .. ..	90 29 8.7	95 33 9.5	.. .. ..	.. .. ..	95 34 9.6
90 32 9.2	100 35 9.4	110 34 9.1	105 32 10.7	90 33 9.9	.. .. ..	80 33 10.6	80 28 10.6	.. .. ..	.. .. ..	83 25 11.1
100 14 9.4	75 11 9.6	100 11 10.2	100 11 9.7	75 12 10.3	.. .. ..	90 14 9.7	87 10 10.4	.. .. ..	.. .. ..	90 10 10.6
75 20 9.1	75 27 8.7	70 24 7.9	65 28 9.0	80 31 9.1	.. .. ..	65 17 9.9	80 13 8.1	.. .. ..	.. .. ..	80 18 9.5
50 4 0 250	50 0 0 0	50 0 0 0	50 4 2 1,730	50 6 0 1,087	.. .. .. ..	50 6 2 570	50 2 0 1,560	.. .. .. ..	.. .. .. ..	.. .. .. ..
0 2 0 0	0 0 0 0	0 0 0 0	280 1 1 0	0 1 2 0	.. .. .. ..	200 1 2 0	0 0 1 0	.. .. .. ..	.. .. .. ..	.. .. .. ..
85 2 10.5	95 1 9.0	100 1 7.0	95 1 9.0	80 4 8.7	.. .. ..	85 1 10.0	90 3 11.0	.. .. ..	.. .. ..	96 2 11.5
50 0 0 0	50 0 0 0	50 0 0 0	50 0 0 0	50 0 0 0	.. .. .. ..	50 0 0 0	50 0 0 0	.. .. .. ..	.. .. .. ..	.. .. .. ..
0 0 0 0	0 0 0 0	0 0 0 0	0 0 0 0	0 0 0 0	.. .. .. ..	0 0 0 0	0 0 .. 0	.. .. .. ..	.. .. .. ..	.. .. .. ..
65 12 9.1	72 6 9.5	60 10 10.8	62 11 10.1	58 10 10.3	.. .. ..	60 12 10.9	65 12 11.3	.. .. ..	.. .. ..	67 21 9.2



TABLE

Name of village.		Jan.	Feb.	Mar.	April.
Chatrapur (Tankakun Tampara huts).	Number of spleen examinations ..	7	..	9	10
	Spleen rate per cent ..	86	..	78	80
	Average enlarged spleen (cm. from umbilicus).	4.2	..	8.7	8.5
Chatrapur (Tampara huts).	Number of spleen examinations ..	11	9	8	6
	Spleen rate per cent ..	64	67	75	67
	Average enlarged spleen (cm. from umbilicus).	8.4	9.3	9.2	9.3
Haripur-Bandra (Gopalpur Creek).	Number of spleen examinations ..	44	..	36	..
	Spleen rate per cent ..	93	..	89	..
	Average enlarged spleen (cm. from umbilicus).	7.2	..	7.8	..
	Number of blood examinations ..	44	..	36	..
	Parasite rate per cent ..	12	..	14	..
	Gametocyte rate per cent ..	2	..	6	..
	Average positive parasite count per c.mm. of blood.	152	..	3,412	..
	Species of parasite { Asexual ..	20	..	160	..
	{ Sexual ..	3	..	4	..
	{ <i>P. falciparum</i> ..	2	..	0	..
	{ <i>P. vivax</i> ..	0	..	1	..
	{ <i>P. malariae</i> ..	0	..	1	..
Gopalpur ..	Number of spleen examinations ..	110	..	115	..
	Spleen rate per cent ..	35	..	30	..
	Average enlarged spleen (cm. from umbilicus).	9.4	..	9.6	..
	Number of blood examinations ..	50	..	50	..
	Parasite rate per cent ..	6	..	4	..
	Gametocyte rate per cent ..	2	..	2	..
	Average positive parasite count per c.mm. of blood.	5,600	..	2,110	..
	Species of parasite { Asexual ..	920	..	80	..
	{ Sexual ..	3	..	1	..
	{ <i>P. falciparum</i> ..	0	..	0	..
	{ <i>P. vivax</i> ..	0	..	1	..
	{ <i>P. malariae</i> ..	0	..	1	..
Uppalputtu (Gopalpur Creek).	Number of spleen examinations ..	..	..	..	..
	Spleen rate per cent ..	..	..	..	..
	Average enlarged spleen (cm. from umbilicus).	..	..	..	..
Pallibonho (near Ganjam).	Number of spleen examinations ..	..	..	..	51
	Spleen rate per cent ..	..	..	..	86
	Average enlarged spleen (cm. from umbilicus).	..	..	..	8.4







TABLE

Name of village.		Jan.	Feb.	Mar.	April.
Boropalli .. (near Ganjam).	Number of spleen examinations ..	..	..	..	21
	Spleen rate per cent ..	..	..	..	95
	Average enlarged spleen (cm. from umbilicus).	..	..	..	9.4
	Number of blood examinations ..	..	..	..	20
	Parasite rate per cent ..	..	..	..	15
	Gametocyte rate per cent ..	..	..	..	0
	Average positive parasite count per c.mm. of blood.	..	..	..	2,953
	Species of parasite	Asexual ..	..	..	0
		Sexual ..	..	..	0
		<i>P. falciparum</i> ..	..	..	3
		<i>P. vivax</i> ..	..	..	0
		<i>P. malariae</i> ..	..	..	0
Ganjam ..	Number of spleen examinations ..	..	..	..	111
	Spleen rate per cent ..	..	..	..	51
	Average enlarged spleen (cm. from umbilicus).	..	..	..	9.0
	Number of blood examinations ..	..	..	..	50
	Parasite rate per cent ..	..	..	..	4
	Gametocyte rate per cent ..	..	..	..	0
	Average positive parasite count per c.mm. of blood.	..	..	..	170
	Species of parasite	Asexual ..	..	..	0
		Sexual ..	..	..	0
		<i>P. falciparum</i> ..	..	..	2
		<i>P. vivax</i> ..	..	..	..
		<i>P. malariae</i> ..	..	..	..
Ganjam .. (Karn Street).	Number of spleen examinations ..	..	..	..	20
	Spleen rate per cent ..	..	..	..	25
	Average enlarged spleen (cm. from umbilicus).	..	..	..	8.4
Kanchipur (near Ganjam).	Number of spleen examinations ..	..	..	..	85
	Spleen rate per cent ..	..	..	..	5
	Average enlarged spleen (cm. from umbilicus).	..	..	..	9.0



IX—conold.

1941.								1942.		
May.	June.	July.	Aug.	Sept.	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.
..	..	23	..	..	..	..	..	..	..	15
..	..	100	..	..	..	..	..	..	..	100
..	..	9·2	..	..	..	..	..	..	..	8·7
..	..	18	..	..	..	..	..	..	..	..
..	..	11	..	..	..	..	..	..	..	..
..	..	11	..	..	..	..	..	..	..	..
..	..	3,600	..	..	..	..	..	..	..	..
..	..	29	..	..	..	..	..	..	..	..
..	..	1	..	..	..	..	..	..	..	..
..	..	1	..	..	..	..	..	..	..	..
..	..	0	..	..	..	..	..	..	..	..
..	..	97	..	..	..	130	..	..	..	124
..	..	35	..	..	..	35	..	..	..	27
..	..	9·7	..	..	..	9·4	..	..	..	8·6
..	..	50	..	..	..	50	..	..	..	..
..	..	0	..	..	..	6	..	..	..	..
..	..	0	..	..	..	0	..	..	..	..
..	..	0	..	..	..	893	..	..	..	..
..	..	0	..	..	..	0	..	..	..	..
..	..	0	..	..	..	3	..	..	..	..
..	..	0	..	..	..	0	..	..	..	..
..	..	0	..	..	..	0	..	..	..	..
..	..	..	..	..	..	12	..	..	..	18
..	..	..	..	..	..	17	..	..	..	11
..	..	..	..	..	..	9	..	..	..	9·5
..	..	..	..	..	..	62	..	..	..	75
..	..	..	..	..	..	3	..	..	..	3
..	..	..	..	..	..	6·9	..	..	..	7·5



TABLE  
Collections of adult anopheline

Year.	Month.	<i>sundaicus.</i>			<i>aconitus.</i>			<i>annularis.</i>			<i>barbirostris.</i>			<i>culicifacies.</i>			<i>hyrcanus.</i>		
		A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C
1939	July	0	0	0	0	0	0	0	43	141	0	0	0	0	8	25	0	0	0
	Aug.	0	0	0	0	0	0	17	160	535	0	0	0	0	18	68	0	0	7
	Sept.	0	0	0	0	0	0	0	27	333	0	0	0	0	7	57	0	0	7
	Oct.	0	0	0	0	0	0	0	215	614	0	0	4	0	6	23	0	1	8
	Nov.	0	0	0	0	0	0	0	566	1,109	0	1	0	0	6	23	0	9	32
	Dec.	0	0	0	0	0	0	0	222	389	0	0	0	0	0	2	0	4	2
1940	Jan.	1	0	0	0	0	0	0	273	150	0	0	0	0	0	1	0	1	4
	Feb.	0	0	0	0	0	0	8	115	17	0	1	0	0	3	0	0	3	1
	March	0	3	0	0	0	0	25	196	68	0	0	0	0	5	3	3	5	3
	April	0	1	0	0	0	0	12	129	43	0	0	0	0	10	2	0	0	0
	May	0	1	0	0	0	0	15	174	60	0	0	0	0	2	0	0	1	1
	June	0	3	0	0	0	0	13	234	86	0	0	0	0	5	1	0	1	0
	July	0	1	0	0	0	0	8	57	31	0	0	0	0	18	11	0	1	1
	Aug.	0	0	0	0	0	0	36	138	85	0	0	0	2	55	26	0	0	0
	Sept.	0	1	0	0	0	0	45	128	70	0	1	1	3	32	19	0	1	1
	Oct.	0	12	6	0	1	0	32	182	114	0	0	0	0	4	2	0	1	2
	Nov.	0	36	16	0	13	8	73	476	253	0	0	1	0	4	4	2	4	9
	Dec.	1	26	23	0	10	4	13	124	49	0	0	1	0	1	0	0	3	6
1941	Jan.	0	94	37	0	0	3	12	84	37	0	0	0	0	0	0	0	4	3
	Feb.	60	339	199	0	1	0	2	29	10	0	0	0	1	3	2	0	0	0
	March	81	263	305	0	0	0	0	1	1	0	0	0	0	0	0	0	1	0
	April	154	225	..	0	0	0	5	1	1	0	0	0	0	0	0	1	0	0
	May	0	1	0	0	0	0	0	7	10	0	0	0	0	0	0	0	0	0
	June	0	0	0	0	0	0	1	27	203	97	0	0	0	0	2	0	0	0
	July	0	0	0	0	0	0	33	322	186	0	0	1	2	13	4	0	0	1
	Aug.	0	0	0	0	0	0	19	144	66	0	0	0	1	18	10	0	0	0
	Sept.	0	11	2	0	0	0	29	194	89	0	0	0	0	6	5	0	0	0
	Oct.	7	18	11	0	0	0	24	132	51	0	0	0	0	2	2	2	4	4
	Nov.	95	390	210	0	4	2	41	232	130	0	0	0	0	0	3	4	9	7
	Dec.	155	626	376	0	1	0	21	104	50	0	0	0	0	0	0	0	2	0
1942	Jan.	157	584	334	0	1	0	10	25	14	0	0	0	0	0	0	0	0	2
	Feb.	37	166	83	0	0	0	1	10	5	0	0	0	0	0	0	0	0	0
	March	42	182	116	0	0	0	1	3	3	0	0	0	0	0	0	0	0	0

A = human dwellings ; B = huts occupied by both



## X-a.

mosquitoes, *Sana Nairi*.

<i>pallidus</i> .			<i>ramsayi</i> .			<i>subpictus</i> .			<i>tessellatus</i> .			<i>vagus</i> .			<i>varuna</i> .			<i>jamesi</i> .		
A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C
0	4	9	0	0	0	0	107	352	0	0	0	0	1	7	0	0	0	0	0	0
0	2	22	0	0	0	0	294	840	0	0	0	0	3	13	0	0	0	0	0	0
0	0	15	0	0	4	0	44	357	0	0	1	0	4	30	0	0	0	0	0	0
0	9	31	0	1	17	0	50	143	0	0	0	0	5	25	0	0	0	0	0	0
0	17	40	0	6	19	0	6	23	0	0	4	0	22	39	0	0	13	0	0	0
0	8	8	0	2	6	0	4	7	0	0	1	0	5	3	0	4	13	0	0	0
0	2	3	0	4	1	0	12	8	0	0	1	0	4	0	0	8	3	0	0	0
0	2	0	0	0	0	1	6	4	0	0	0	0	0	0	0	8	0	0	0	0
0	7	2	0	4	0	11	25	15	0	1	0	7	13	13	0	9	2	0	0	0
0	1	0	0	0	0	17	53	21	0	0	0	0	3	1	0	3	0	0	0	0
0	1	0	0	0	0	17	76	31	0	0	0	0	2	2	0	0	0	0	0	0
0	11	3	0	0	0	29	144	73	0	0	0	0	9	4	0	0	0	0	0	0
0	4	1	0	0	0	42	376	227	0	0	0	3	10	12	0	2	0	0	0	0
0	1	0	0	3	0	38	388	212	0	0	1	17	59	44	0	3	1	0	0	0
0	3	0	0	2	3	26	105	53	0	0	0	20	47	37	0	5	2	0	0	0
0	0	2	0	5	9	17	63	28	0	0	1	11	34	34	0	4	1	0	0	1
0	0	1	0	3	1	9	20	20	0	2	0	7	10	8	0	5	3	0	0	0
0	2	1	0	2	1	11	31	13	0	0	0	0	1	2	0	2	2	0	0	0
0	1	0	0	1	1	19	89	57	0	0	0	0	1	3	0	2	1	0	0	0
0	0	0	0	0	1	30	102	83	0	0	1	0	2	5	0	0	0	0	0	0
0	0	0	0	0	0	120	230	276	0	0	0	0	0	10	0	0	0	0	0	0
0	0	0	0	0	0	137	268	6	0	0	0	6	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	62	32	0	0	0	0	0	0	0	0	0	0	0	0
0	1	0	0	0	0	41	235	143	0	0	0	0	0	0	0	0	0	0	0	0
0	0	1	0	0	0	149	572	382	0	0	0	1	0	1	0	0	0	0	0	0
0	3	3	0	0	0	171	413	255	0	0	0	0	0	0	0	0	0	0	0	0
2	28	17	0	0	1	170	887	430	0	0	0	0	0	0	0	0	0	0	0	0
0	9	8	0	15	0	152	739	466	0	0	0	1	6	7	0	0	0	0	1	0
3	17	10	0	1	2	173	595	382	0	0	1	0	0	0	0	1	1	0	0	0
0	4	3	0	0	0	84	245	155	0	0	0	0	0	0	0	0	0	0	0	0
0	0	1	0	1	0	84	221	165	0	0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	1	32	65	42	0	0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	39	122	72	0	0	0	0	0	0	0	0	0	0	0	0

human beings and cattle; C = cattle-sheds.



TABLE

Collections of adult anopheline

Year.	Month.	sundaicus.			aconitus.			annularis.			barbirostris.			culicifacies.			hyrcanus.		
		A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C
1939	May ..	0	0	0	0	0	0	1	3	0	0	0	0	0	0	0	0	0	0
	June ..	0	0	0	0	0	0	3	4	0	0	0	0	0	0	0	0	0	0
	July ..	0	0	0	0	0	0	2	31	63	0	0	0	1	8	22	0	0	1
	Aug. ..	0	0	0	0	0	0	0	59	174	0	0	0	0	19	65	0	0	0
	Sept. ..	0	0	0	0	0	0	1	10	67	0	0	0	0	7	35	0	0	2
	Oct. ..	0	0	0	0	0	0	3	49	224	0	0	9	0	3	25	2	0	9
	Nov. ..	0	0	0	0	0	0	29	240	477	0	3	19	0	0	13	8	2	27
	Dec. ..	0	0	0	0	0	0	9	100	141	0	0	4	0	3	6	0	3	5
1940	Jan. ..	0	0	0	0	0	0	4	58	27	0	0	0	1	6	1	0	0	1
	Feb. ..	0	0	0	0	0	0	1	21	2	0	0	0	0	1	0	0	1	0
	March ..	0	0	0	0	0	0	0	11	4	0	0	0	0	21	4	0	2	0
	April ..	0	1	0	0	0	0	0	8	2	0	0	0	0	22	3	0	0	1
	May ..	0	0	0	0	0	0	0	14	4	0	0	0	0	2	1	0	0	0
	June ..	0	0	0	0	0	0	0	31	12	0	0	0	0	17	5	0	0	0
	July ..	0	0	0	0	0	0	2	21	9	0	0	0	0	33	10	0	0	1
	Aug. ..	0	0	0	0	0	0	0	22	8	0	0	0	0	23	21	0	1	0
	Sept. ..	0	0	0	0	0	0	0	19	4	0	0	0	5	61	30	0	2	1
	Oct. ..	0	0	0	0	0	0	6	79	47	0	0	0	0	22	8	0	1	3
	Nov. ..	0	1	0	1	38	24	15	267	159	0	5	2	0	22	14	0	4	3
	Dec. ..	0	0	0	0	6	3	0	14	4	0	0	2	0	4	1	0	0	0
1941	Jan. ..	0	0	0	0	1	0	0	2	2	0	0	0	0	2	4	0	0	1
	Feb. ..	0	1	0	0	0	0	0	6	3	0	0	0	0	3	1	0	0	0
	March ..	0	8	2	0	0	0	0	3	7	0	0	0	0	4	2	0	0	0
	April ..	0	2	0	0	0	0	0	1	3	0	0	0	0	0	0	0	0	0
	May ..	0	0	0	0	0	0	0	2	1	0	0	0	0	0	0	0	0	0
	June ..	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	July ..	0	0	0	0	0	0	1	41	20	0	0	1	1	18	11	0	0	0
	Aug. ..	0	0	0	0	0	0	3	52	24	0	0	0	0	2	2	0	0	0
	Sept. ..	0	0	0	0	0	0	3	56	26	0	0	0	0	3	2	0	0	1
	Oct. ..	0	0	0	0	0	0	13	85	41	0	0	0	0	0	0	0	3	2
	Nov. ..	0	3	0	0	1	0	14	142	86	0	0	0	0	1	5	0	0	1
	Dec. ..	0	3	0	1	6	4	9	46	28	0	0	0	1	3	3	0	0	2
1942	Jan. ..	0	0	0	0	2	0	11	56	27	0	0	0	0	1	4	0	3	1
	Feb. ..	0	2	0	0	0	0	2	19	13	0	0	0	0	1	3	0	0	0
	March ..	0	0	0	0	0	0	0	2	1	0	0	0	0	0	0	0	0	0

A = human dwellings; B = huts occupied by both



## X-b.

mosquitoes, *Balugaon*.

<i>pallidus.</i>			<i>ramayyi.</i>			<i>subpictus.</i>			<i>tessellatus.</i>			<i>vagus.</i>			<i>varuna.</i>			<i>fluviatilis.</i>			<i>maculatus.</i>		
A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C
0	0	0	0	0	0	8	31	46	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	96	125	35	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
1	0	3	0	0	0	57	226	623	0	0	0	0	1	13	0	0	0	0	0	0	0	0	1
0	0	10	0	0	0	0	277	875	0	0	0	0	3	10	0	0	0	0	0	0	0	0	0
0	0	5	0	0	0	2	20	230	0	0	0	0	0	17	0	0	1	0	0	0	0	0	0
0	0	14	0	0	1	7	37	168	0	0	0	0	1	23	0	0	1	0	0	0	0	0	0
0	3	22	0	1	5	3	5	22	0	0	1	0	2	18	0	0	10	0	0	0	0	0	0
1	8	9	0	2	3	15	10	14	0	2	5	6	5	3	0	16	18	0	0	0	0	0	0
0	2	0	0	0	0	4	36	4	0	0	6	2	1	1	0	5	2	0	0	0	0	0	0
0	0	0	0	0	1	0	8	38	12	0	0	0	0	1	0	1	0	0	0	0	0	0	0
0	3	0	0	0	0	17	89	38	0	0	0	2	5	2	0	3	0	0	0	0	0	0	0
0	1	0	0	2	0	8	103	20	0	0	0	1	5	1	0	0	0	0	0	0	0	0	0
0	5	1	0	0	0	0	38	11	0	0	0	0	3	1	0	0	0	0	0	0	0	0	0
0	8	0	0	0	0	14	146	65	0	0	0	0	11	4	0	0	0	0	0	0	0	0	0
0	5	0	0	0	0	87	570	302	0	0	0	4	20	12	0	0	0	0	0	0	0	0	0
0	2	1	0	0	0	74	410	270	0	0	0	15	26	28	0	1	0	0	0	0	0	0	0
0	0	0	0	2	0	37	174	93	0	0	0	24	46	36	0	0	0	0	0	0	0	0	0
0	0	1	0	2	2	22	87	79	0	0	0	8	48	40	0	3	2	0	0	0	0	0	0
0	3	1	0	4	6	27	142	92	0	1	0	9	32	27	0	4	3	0	0	0	0	0	0
0	0	0	0	1	0	9	83	43	0	1	0	0	1	1	0	0	0	0	0	0	0	0	0
0	0	0	0	1	0	4	74	46	0	0	0	0	1	0	0	1	0	0	1	0	0	0	0
0	0	0	0	0	0	11	68	40	0	1	0	0	1	5	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	61	192	123	0	0	0	1	1	1	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	13	64	76	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	3	47	30	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	33	155	95	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
0	0	1	0	0	0	51	297	230	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
0	2	3	0	0	0	39	283	188	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
0	8	5	0	0	1	69	346	191	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
3	6	6	0	0	0	69	340	256	0	0	0	3	5	2	0	0	0	0	0	0	0	0	0
2	8	9	0	0	0	73	284	189	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
1	5	10	0	0	0	67	249	132	0	1	0	0	0	0	0	1	0	0	0	0	0	0	0
0	1	1	0	0	0	76	351	200	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0
0	0	0	0	0	0	66	402	227	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	28	99	55	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0

human beings and cattle; C = cattle-sheds.



TABLE

Collections of adult anopheline

Year.	Month.	sundaicus.			aconitus.			annularis.			barbistrois.			culicifacies.			hyrcanus.		
		A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C
1939	June ..	0	0	0	0	0	0	1	0	3	0	0	0	0	0	0	0	0	0
	July ..	0	0	0	0	0	0	11	11	5	0	0	0	6	4	2	0	1	0
	Aug. ..	0	0	0	0	0	0	44	55	27	0	0	0	19	24	20	0	2	0
	Sept. ..	0	0	0	0	0	0	32	19	36	0	0	0	6	6	16	0	0	0
	Oct. ..	1	0	0	0	0	0	22	9	16	0	0	0	13	6	8	0	0	15
	Nov. ..	0	0	0	0	0	0	41	37	110	0	0	0	3	4	3	5	11	127
	Dec. ..	0	0	0	0	0	0	24	10	63	0	0	0	0	0	0	0	0	10
1940	Jan. ..	0	0	0	0	0	0	0	2	8	0	0	0	0	0	0	0	0	0
	Feb. ..	0	0	0	0	0	0	2	5	8	0	0	0	0	0	0	0	0	0
	March ..	0	0	1	0	0	0	0	5	32	0	0	1	0	0	0	0	0	4
	April ..	0	0	1	0	0	0	5	17	49	0	3	1	0	0	0	4	8	4
	May ..	3	0	0	0	0	0	18	23	44	2	2	1	0	0	0	2	2	5
	June ..	0	0	0	0	0	0	59	62	91	0	1	1	0	0	0	1	1	0
	July ..	0	0	0	0	0	0	68	83	107	0	0	0	14	10	34	0	1	1
	Aug. ..	121	0	4	0	0	0	19	16	45	0	1	2	16	5	15	1	2	0
	Sept. ..	1,186	0	92	0	0	0	5	0	2	0	0	0	13	0	2	0	0	2
	Oct. ..	1,947	0	122	0	0	0	11	0	4	0	0	0	2	0	0	0	0	0
	Nov. ..	866	0	84	6	0	3	3	0	0	0	0	0	0	0	0	0	1	4
	Dec. ..	615	16	55	3	0	2	1	1	2	0	0	0	0	0	0	4	1	8
1941	Jan. ..	458	11	39	2	0	0	4	1	10	0	0	0	0	0	1	1	0	4
	Feb. ..	71	0	8	1	0	2	2	0	18	0	0	0	0	0	1	0	0	3
	March ..	13	0	0	0	0	2	6	0	49	0	0	0	0	0	2	0	0	0
	April ..	0	0	0	0	0	0	7	0	50	0	0	0	0	0	0	0	0	1
	May ..	0	0	0	0	0	0	0	0	58	0	0	0	0	0	0	0	0	0
	June ..	1	0	0	0	0	0	16	0	159	0	0	0	2	0	0	0	0	0
	July ..	0	0	0	0	0	0	8	0	85	0	0	0	1	0	5	0	0	0
	Aug. ..	0	0	0	0	0	0	3	0	43	0	0	0	1	0	6	0	0	0
	Sept. ..	0	0	0	0	0	0	2	0	33	0	0	0	2	0	6	0	0	9
	Oct. ..	20	0	0	0	0	0	6	0	43	0	0	0	0	0	1	0	1	2
	Nov. ..	53	0	29	1	0	0	15	7	90	0	0	0	1	0	0	0	0	33
	Dec. ..	69	0	65	0	0	1	6	9	104	0	0	0	0	0	1	0	3	16
1942	Jan. ..	14	0	27	0	0	1	8	14	89	0	0	0	0	0	0	0	0	4
	Feb. ..	8	0	17	0	0	0	0	15	129	0	0	0	0	0	0	0	2	5
	March ..	17	0	18	0	0	1	2	14	89	0	0	0	0	0	0	0	0	5

A = human habitations ; B = huts occupied by both



X-c.

mosquitoes, Kesopur.

<i>pallidus.</i>			<i>ramsayi.</i>			<i>subpictus.</i>			<i>tessellatus.</i>			<i>vagus.</i>			<i>varuna.</i>			<i>jamesi.</i>		
A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C
0	0	0	0	0	0	62	55	68	0	0	0	0	0	0	0	0	0	0	0	0
1	0	0	0	0	0	211	216	243	0	0	0	1	0	0	0	0	0	0	0	0
5	3	5	0	0	0	284	300	336	0	0	0	3	5	6	0	0	0	0	0	0
0	0	5	0	0	0	246	355	298	0	0	0	2	1	3	0	0	0	0	0	0
1	1	4	0	0	0	68	65	71	0	0	0	3	0	2	0	0	0	0	0	0
2	0	21	0	0	0	82	34	212	0	0	1	4	8	28	1	0	12	0	0	0
0	0	3	0	0	0	29	36	97	0	0	0	0	0	5	0	0	8	0	0	0
0	0	0	0	0	0	12	8	33	0	0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	5	8	29	0	0	0	0	0	0	0	0	0	0	0	0
0	1	0	0	0	0	3	26	75	0	0	0	0	1	1	0	0	0	0	0	0
0	0	0	0	0	0	26	40	55	0	0	0	0	0	0	0	0	0	0	0	0
0	1	3	0	0	0	51	73	111	0	0	0	0	0	0	0	0	0	0	0	0
0	2	13	0	0	0	195	139	194	0	0	0	1	0	1	0	0	0	0	0	0
11	12	19	0	0	5	365	216	368	0	0	0	13	7	12	0	0	0	0	0	0
0	1	3	0	0	0	1,070	341	588	0	0	0	131	50	79	0	0	0	0	0	0
0	0	0	3	0	0	1,240	229	643	0	0	0	499	76	84	0	0	0	0	0	0
0	0	0	2	0	0	1,097	147	418	0	0	0	300	34	208	0	0	0	0	0	0
0	0	0	2	0	0	241	145	249	0	0	0	78	30	154	0	0	0	0	0	0
0	0	0	5	0	0	101	66	94	0	0	0	19	14	29	0	0	0	0	0	0
0	0	0	0	0	0	492	110	263	0	0	0	21	8	11	0	0	0	0	0	0
0	0	0	0	0	2	281	58	253	0	0	0	13	4	7	0	0	0	0	0	0
0	0	0	0	0	0	420	131	248	0	0	0	35	8	25	0	0	0	0	0	0
0	0	0	0	0	0	63	23	43	0	0	0	7	1	6	0	0	0	0	0	1
0	0	0	0	0	0	31	2	12	0	0	0	0	0	0	0	0	0	0	0	2
0	0	0	0	0	0	170	24	47	0	0	0	11	2	7	0	0	0	0	0	0
0	0	0	0	0	0	150	28	93	0	0	0	20	4	4	0	0	0	0	0	0
0	0	2	0	0	0	231	42	266	0	0	0	21	10	26	0	0	0	0	0	0
0	0	0	0	0	0	118	30	200	0	0	1	17	6	26	0	0	0	0	0	0
0	0	10	0	0	0	127	26	233	0	0	0	26	6	30	0	0	0	0	0	0
6	2	23	0	0	0	303	60	652	0	0	0	36	12	72	0	0	0	0	0	0
1	3	9	0	0	0	211	76	620	0	0	0	22	12	55	0	0	0	0	0	0
0	0	9	0	0	0	119	36	346	0	0	0	11	2	33	0	0	0	0	0	0
0	0	19	0	0	0	118	38	331	0	0	0	7	2	23	0	0	0	0	0	0
0	0	13	0	0	0	42	22	117	0	0	0	5	1	11	0	0	0	0	0	0

human beings and cattle; C = cattle-sheds.

J, MI



TABLE  
Collections of adult anopheline

Year.	Month.	<i>sundaicus.</i>			<i>aconitus.</i>			<i>annularis.</i>			<i>barbirostris.</i>			<i>culicifacies.</i>		
		A	B	C	A	B	C	A	B	C	A	B	C	A	B	C
1939	May ..	3	0	1	0	0	0	0	0	2	0	0	0	0	0	0
	June ..	1	0	0	0	0	0	2	4	2	0	0	0	0	1	0
	July ..	0	0	0	0	0	0	0	7	8	0	0	0	0	4	4
	Aug. ..	0	0	0	0	0	0	2	70	47	0	0	0	0	25	5
	Sept. ..	0	0	0	0	0	0	6	9	22	0	0	0	0	5	7
	Oct. ..	0	0	0	0	0	0	4	42	146	0	0	7	0	2	6
	Nov. ..	0	0	0	0	0	6	15	89	391	0	4	48	0	2	29
	Dec. ..	0	0	0	0	1	8	0	48	377	0	2	18	0	1	5
1940	Jan. ..	0	0	0	0	0	0	7	41	232	1	3	5	0	0	0
	Feb. ..	0	1	1	0	0	0	3	15	73	0	1	3	0	0	1
	March ..	0	3	12	0	0	0	0	5	60	0	0	1	0	0	1
	April ..	0	5	19	0	0	0	12	25	73	0	0	1	0	0	1
	May ..	1	0	0	0	0	0	19	21	47	1	1	1	0	0	0
	June ..	5	0	0	0	0	0	81	59	96	0	0	0	3	0	3
	July ..	0	0	0	0	0	0	21	21	37	0	0	0	13	14	33
	Aug. ..	0	0	0	0	0	0	16	10	28	0	0	0	25	13	32
	Sept. ..	9	2	7	0	0	0	14	7	18	0	0	0	17	15	20
	Oct. ..	16	5	18	0	0	0	5	15	19	0	0	0	0	1	4
	Nov. ..	39	36	68	0	0	0	22	42	65	0	0	0	0	0	0
	Dec. ..	9	23	36	0	0	0	15	24	35	0	0	0	0	0	0
1941	Jan. ..	7	17	31	0	0	0	1	2	9	0	0	0	0	0	1
	Feb. ..	2	1	5	0	0	0	0	0	0	0	0	0	0	0	0
	March ..	0	2	1	0	0	0	0	0	0	0	0	0	0	0	0
	April ..	1	8	0	0	0	0	0	0	1	0	0	0	0	0	0
	May ..	0	0	0	0	0	0	0	0	5	0	0	0	0	0	0
	June ..	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
	July ..	0	0	0	0	0	0	1	1	2	0	0	0	0	1	4
	Aug. ..	0	0	0	0	0	0	2	3	7	0	0	0	1	2	0
	Sept. ..	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Oct. ..	0	0	0	0	0	0	22	28	46	0	0	0	0	3	0
	Nov. ..	0	0	0	2	0	0	74	105	174	0	2	4	2	1	0
	Dec. ..	2	5	2	4	9	3	5	17	24	0	0	1	0	1	0
1942	Jan. ..	0	1	0	1	6	3	6	22	34	0	0	0	0	2	0
	Feb. ..	0	0	0	0	0	0	0	12	16	0	0	0	0	0	0
	March ..	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0

A = human habitations ; B = huts occupied by both



X-d.

mosquitoes, Konoka.

hyrcanus.			pallidus.			ramsayi.			subpictus.			tessellatus.			vagus.			varuna.		
A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C
0	0	0	0	0	0	0	0	0	26	26	35	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	29	45	36	0	0	0	0	2	0	0	0	0
0	0	0	0	1	0	0	0	0	44	166	73	0	0	0	0	3	1	0	0	0
0	0	0	0	3	1	0	0	0	21	269	218	0	0	0	4	4	3	0	0	0
0	0	1	2	0	2	0	0	0	45	47	71	0	0	0	0	1	0	0	0	1
0	0	4	0	0	4	0	0	1	17	30	89	0	0	0	1	11	29	0	0	0
0	1	26	0	4	45	0	0	0	20	70	344	0	0	5	6	7	32	0	5	26
1	0	3	0	1	27	0	0	0	16	73	295	0	0	1	0	1	3	1	12	58
0	1	1	1	3	5	0	0	0	29	102	243	0	0	0	0	0	7	0	3	12
0	0	2	0	0	0	0	0	0	11	38	294	0	0	0	0	0	2	0	0	13
0	0	1	0	0	0	0	0	0	13	35	147	0	0	0	0	2	0	0	0	4
1	1	2	0	2	5	0	0	0	51	46	143	0	0	0	0	0	0	0	0	0
0	1	1	0	0	0	0	0	0	63	74	171	0	0	0	0	0	0	0	0	0
0	0	1	0	1	3	0	0	0	250	154	256	0	0	0	0	0	4	0	0	0
0	1	2	2	1	8	0	0	0	343	322	487	0	0	0	22	29	40	0	0	0
0	2	21	0	1	3	0	0	0	446	341	607	0	0	0	46	45	68	0	0	0
1	3	9	0	0	0	0	0	0	242	224	342	0	0	0	76	50	73	0	0	0
0	7	14	0	0	0	0	0	0	323	376	790	0	0	1	76	72	164	0	0	0
2	16	26	0	0	0	0	0	0	232	221	568	0	0	0	41	53	116	0	0	0
2	7	8	0	0	0	0	0	0	109	130	238	0	0	0	17	15	33	0	0	0
0	0	4	0	0	0	0	0	0	220	249	586	0	0	0	15	18	31	0	0	0
0	0	0	0	0	0	0	0	0	37	42	110	0	0	0	2	1	3	0	0	0
0	0	0	0	0	0	0	0	0	14	11	45	0	0	0	2	4	3	0	0	0
0	0	0	0	0	0	0	0	0	7	26	13	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	2	6	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	3	11	13	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	12	15	32	0	0	0	0	0	0	0	0	0
0	0	0	1	0	0	0	0	0	22	44	27	0	0	0	1	2	0	0	0	0
0	0	0	0	0	0	0	0	0	17	15	20	0	0	0	3	2	4	0	0	0
0	3	2	0	6	5	0	0	0	24	22	20	0	0	0	7	6	12	0	0	0
0	0	2	0	26	44	0	0	0	113	64	132	0	0	0	16	13	22	0	0	0
0	0	2	0	6	8	0	0	0	95	64	90	0	0	0	90	13	13	0	0	0
0	2	2	0	7	13	0	0	0	357	293	446	0	0	0	45	36	57	0	0	0
0	0	0	0	0	0	0	0	0	38	57	80	0	0	0	2	3	1	0	0	0
0	0	0	0	0	0	0	0	0	3	15	28	0	0	0	0	0	2	0	0	0

human beings and cattle; C = cattle-sheds.



TABLE  
Collections of adult anopheline

Year.	Month.	<i>sundaicus.</i>			<i>aconitus.</i>			<i>annularis.</i>			<i>barbirostris.</i>			<i>culicifacies.</i>			<i>hyrcanus.</i>		
		A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C
1939	April ..	61	9	42	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0
	May ..	196	12	83	0	0	0	2	0	5	0	0	0	0	0	0	0	1	0
	June ..	0	0	2	0	0	0	2	3	2	0	0	0	1	0	0	0	0	0
	July ..	0	0	0	0	0	0	1	5	10	0	0	0	10	10	2	0	0	..
	Aug. ..	0	0	0	0	0	0	6	36	28	0	1	0	42	44	14	2	0	1
	Sept. ..	0	0	0	0	0	0	3	22	1	0	0	0	43	27	6	0	0	0
	Oct. ..	0	0	1	0	0	0	1	34	18	0	1	1	5	5	3	0	1	3
	Nov. ..	0	0	0	0	0	0	0	56	15	0	6	3	2	2	8	0	15	13
	Dec. ..	0	0	0	0	0	0	0	35	2	0	2	0	0	3	0	0	0	0
1940	Jan. ..	0	2	0	0	0	0	1	14	20	0	0	0	0	0	0	0	1	2
	Feb. ..	2	7	15	0	0	0	0	24	44	0	0	2	0	0	1	0	1	0
	March ..	160	75	88	0	0	0	5	11	80	0	0	2	3	0	1	5	8	1
	April ..	498	126	186	0	0	0	37	50	98	0	0	1	3	1	1	1	4	3
	May ..	23	15	6	0	0	0	14	29	42	1	0	0	0	0	0	0	0	2
	June ..	4	0	0	0	0	0	8	49	39	0	0	1	0	3	4	0	0	0
	July ..	0	0	0	0	0	0	4	54	44	0	1	1	19	20	23	0	0	0
	Aug. ..	0	0	0	0	0	0	4	8	17	0	0	0	22	11	23	0	1	1
	Sept. ..	1	0	0	0	0	0	6	8	11	0	0	0	10	2	25	0	2	0
	Oct. ..	36	9	7	0	1	0	10	13	26	0	0	0	0	0	1	0	0	4
	Nov. ..	62	9	2	0	1	1	6	24	33	0	0	0	0	0	1	1	11	2
	Dec. ..	5	12	2	0	0	3	0	9	11	0	0	0	0	0	0	0	3,	9
1941	Jan. ..	33	34	43	0	1	0	8	32	38	0	3	0	0	0	0	74	11	15
	Feb. ..	16	14	38	3	1	0	4	7	11	0	0	0	1	0	1	14	12	13
	March ..	23	18	16	0	2	0	5	24	30	0	0	0	1	1	0	30	26	26
	April ..	3	1	0	0	0	0	0	1	7	0	0	0	0	0	0	8	7	10
	May ..	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	June ..	0	0	0	0	0	0	0	2	1	0	0	0	0	0	0	0	0	0
	July ..	0	0	0	0	0	0	0	9	5	0	0	0	1	3	6	0	0	0
	Aug. ..	0	0	0	0	0	0	16	32	56	0	0	0	9	11	12	0	3	3
	Sept. ..	1	0	0	0	0	0	17	34	51	1	1	0	6	12	17	0	3	1
	Oct. ..	0	0	0	1	0	0	3	17	19	0	1	0	2	4	0	0	6	4
	Nov. ..	4	1	1	2	0	0	7	42	71	1	0	3	1	1	0	0	3	1
	Dec. ..	2	0	1	6	7	2	2	18	32	0	0	1	1	1	0	0	3	4
1942	Jan. ..	0	1	1	0	0	3	2	44	41	0	1	1	0	1	0	1	2	3
	Feb. ..	0	0	0	0	1	2	1	50	32	0	0	0	0	1	1	0	0	0
	March ..	0	0	0	0	0	0	0	24	12	0	0	0	0	0	0	0	0	0

A = human habitations ; B = huts occupied by both



X-c.

mosquitoes, *Sabilia*.

<i>pallidus.</i>			<i>ramsayi.</i>			<i>subpictus.</i>			<i>tesellatus.</i>			<i>vagus.</i>			<i>varuna.</i>			<i>fluviatilis.</i>		
A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C
0	0	0	0	..	0	27	9	8	0	0	0	0	1	0	0	0	0	0	0	0
0	0	0	0	..	0	284	33	225	0	0	0	5	0	0	0	0	0	0	0	0
0	0	0	0	..	0	103	63	30	0	0	0	0	0	0	0	0	0	0	0	0
1	3	0	0	0	0	394	352	111	0	0	0	2	2	0	0	0	0	0	0	0
..	..	..	..	..	..	566	638	425	..	..	..	2	2	4	0	0	0	0	0	0
4	4	0	0	0	0	343	274	184	0	0	0	6	9	12	0	0	0	0	0	0
0	0	0	0	0	0	60	68	57	0	0	0	14	34	17	1	1	0	0	0	0
0	4	3	0	0	3	20	44	43	0	1	2	2	19	15	0	1	0	0	0	0
0	2	0	0	0	1	0	35	8	0	0	0	1	3	0	0	2	0	0	0	0
0	1	0	0	0	0	0	58	29	0	0	0	0	1	0	0	1	0	0	0	0
0	0	3	0	1	2	6	90	183	0	0	0	0	0	0	0	1	0	0	0	0
0	0	2	1	4	3	520	375	541	1	0	0	3	7	3	1	1	4	0	0	0
1	0	2	0	0	0	967	595	552	0	0	0	3	1	0	2	0	0	0	0	0
2	2	3	0	0	0	403	371	325	0	0	0	1	0	0	2	0	0	0	0	0
2	4	7	0	0	0	251	203	218	0	0	0	4	5	6	2	0	0	0	0	0
0	6	5	0	0	0	350	275	360	0	0	0	19	21	31	0	1	0	0	0	0
0	0	0	0	0	1	503	263	426	0	0	0	52	45	81	0	0	1	0	0	0
1	0	0	1	1	0	238	93	143	0	0	0	101	27	38	0	0	0	0	0	0
0	0	0	2	0	0	421	191	421	0	0	0	34	15	24	1	0	0	0	0	0
0	0	0	0	1	4	216	129	213	0	0	0	16	2	3	0	0	0	0	0	0
0	0	0	0	0	3	24	49	109	0	0	0	0	1	2	0	0	0	0	0	0
1	0	1	1	0	3	243	310	374	0	0	0	0	0	2	0	0	0	0	0	0
0	0	0	0	0	0	103	84	140	0	0	0	0	1	0	0	0	0	0	0	1
0	0	0	0	0	0	97	116	98	0	0	0	1	0	0	0	0	0	0	0	0
0	1	1	0	0	0	23	22	26	0	0	0	0	0	0	0	0	1	0	0	0
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	4	13	21	0	0	0	1	2	4	0	0	0	0	0	0
0	0	0	0	0	0	114	79	113	0	0	0	13	10	15	0	0	0	0	0	0
1	2	4	0	0	0	715	458	707	0	0	0	64	47	67	0	0	0	0	0	0
2	4	8	0	0	0	465	328	494	0	0	0	78	50	81	0	0	0	0	0	0
1	10	6	0	0	0	122	109	135	0	0	0	16	18	18	0	0	0	0	0	0
2	20	25	0	0	0	92	50	84	0	0	0	8	9	9	1	0	0	0	0	0
1	3	5	0	0	0	68	35	62	0	2	4	1	3	8	0	0	0	0	0	0
0	3	0	0	0	0	49	139	128	0	0	0	0	3	5	0	0	1	0	0	0
0	0	0	0	0	0	4	28	21	0	0	0	0	3	1	0	1	1	0	0	0
0	0	0	0	0	0	3	8	8	0	0	0	0	1	0	0	0	1	0	0	0

human beings and cattle; C = cattle-sheds.



TABLE  
Collections of adult anopheline

Year.	Month.	sundaicus.			aconitus.			annularis.			barbirostris.			culicifacies.			hyrcanus.		
		A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C
1939	May ..	1	0	0	0	0	0	1	3	0	0	0	0	0	0	0	0	0	0
	June ..	0	0	0	0	0	0	10	31	44	0	0	0	8	1	5	0	0	0
	July ..	0	0	0	0	0	0	21	73	114	0	0	0	19	11	16	0	0	0
	Aug. ..	0	0	0	0	0	0	11	136	285	0	0	0	10	33	33	0	0	0
	Sept. ..	0	0	0	0	0	0	7	15	26	0	0	0	24	4	38	0	1	0
	Oct. ..	0	0	0	0	0	0	6	24	24	0	0	0	3	2	5	0	4	0
	Nov. ..	0	0	0	0	0	0	6	130	96	0	6	1	4	16	14	0	17	20
	Dec. ..	0	0	0	0	0	0	0	58	59	0	1	0	0	2	0	0	5	9
1940	Jan. ..	2	1	2	0	0	0	0	38	90	0	0	0	0	0	0	0	2	5
	Feb. ..	..	..	..	0	0	0	0	18	8	0	0	0	0	1	1	0	1	0
	March ..	0	4	0	0	0	0	0	23	3	0	0	0	0	1	0	0	0	5
	April ..	0	7	5	0	0	0	9	57	43	0	0	0	0	4	2	0	1	0
	May ..	4	5	0	0	0	0	4	51	22	0	0	0	0	0	1	0	0	1
	June ..	8	6	1	0	0	0	2	16	16	0	0	0	2	2	4	0	0	0
	July ..	1	2	1	0	0	0	10	27	49	0	0	0	8	7	26	0	1	2
	Aug. ..	4	1	0	0	0	0	3	20	41	0	0	0	5	11	7	0	1	3
	Sept. ..	1	1	0	0	0	0	3	40	14	0	0	0	5	25	73	0	0	2
	Oct. ..	29	21	3	0	1	0	7	191	41	0	0	3	0	4	6	0	6	24
	Nov. ..	14	18	0	1	7	7	3	92	62	0	0	0	0	1	0	2	14	22
	Dec. ..	9	9	10	1	7	7	1	8	16	0	0	0	0	1	0	1	3	11
1941	Jan. ..	9	9	4	0	4	4	5	13	38	0	0	0	0	0	0	1	2	1
	Feb. ..	4	2	1	1	1	2	6	13	21	0	0	0	1	0	0	0	1	4
	March ..	6	1	1	0	0	0	6	45	99	0	0	0	1	0	1	0	0	0
	April ..	0	0	0	0	0	0	1	14	55	0	0	0	0	0	0	0	0	0
	May ..	0	0	0	0	0	0	0	4	35	0	0	0	0	0	0	0	0	0
	June ..	0	0	1	0	0	0	28	6	138	0	0	0	1	0	0	0	0	0
	July ..	1	0	0	0	0	0	1	11	9	0	0	0	1	5	0	0	0	0
	Aug. ..	0	0	0	0	0	0	1	35	4	0	0	0	3	10	0	0	0	0
	Sept. ..	0	0	0	0	0	0	0	22	1	0	0	0	2	3	0	0	0	0
	Oct. ..	0	0	0	0	0	0	2	8	16	0	0	0	1	2	0	0	1	3
	Nov. ..	2	5	0	0	0	0	14	65	90	0	1	0	1	0	1	2	7	10
	Dec. ..	1	2	0	0	0	0	9	34	45	0	0	0	0	0	0	0	8	15
1942	Jan. ..	0	1	0	0	0	0	2	36	39	0	0	0	0	0	0	0	7	4
	Feb. ..	0	1	0	0	0	0	9	68	96	0	0	0	0	1	0	0	3	9
	March ..	0	0	0	0	0	0	0	10	17	0	0	0	0	0	0	0	0	0

A = human habitations ; B = huts occupied by both



X-f.

mosquitoes, Tolo Humma.

pallidus.			ramsayi.			subpictus.			tessellatus.			vagus.			varuna.		
A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C
0	0	0	0	0	0	20	11	17	0	0	0	1	0	0	0	0	0
0	0	0	0	0	0	207	145	223	0	0	0	1	1	1	0	0	0
1	0	1	0	0	0	536	460	405	0	0	0	13	6	1	0	0	0
1	1	5	0	0	0	678	585	497	0	0	0	40	28	32	0	0	0
1	1	1	0	0	0	221	164	173	0	0	0	15	27	18	0	0	0
0	2	0	2	12	2	52	66	57	0	0	0	58	38	34	0	0	0
8	9	8	44	59	36	82	95	64	0	0	1	55	46	37	4	16	8
0	12	6	0	9	10	2	43	36	0	0	0	1	13	10	0	3	6
0	1	2	0	1	2	0	8	30	0	0	0	0	3	5	0	2	0
0	0	0	0	0	0	0	15	38	0	0	0	0	0	4	0	1	0
0	1	0	0	0	0	0	2	10	0	0	0	0	1	4	0	1	0
0	3	0	0	0	0	17	17	21	0	0	0	0	4	1	0	0	1
0	2	2	0	0	0	6	34	131	0	0	0	0	1	0	0	0	0
3	9	4	0	0	0	382	302	431	0	0	0	0	3	6	0	0	0
0	4	8	0	0	0	710	421	743	0	0	0	35	28	55	0	0	1
0	0	4	0	5	5	447	273	537	0	0	0	119	57	95	0	0	0
0	1	1	0	0	0	270	139	314	0	0	0	244	141	398	0	0	0
0	0	1	0	0	0	153	84	305	0	0	0	110	18	79	0	0	0
0	0	0	0	0	1	126	69	161	0	0	0	35	12	6	0	0	1
0	1	0	0	0	1	36	21	38	0	0	0	10	5	5	0	0	0
0	0	0	0	0	1	28	16	33	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	37	26	70	0	0	0	0	4	1	0	0	0
0	0	0	0	0	0	36	30	24	0	0	0	0	1	1	0	0	0
0	0	0	0	0	0	14	11	23	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	1	1	9	0	0	0	0	0	1	0	0	0
0	0	0	0	0	0	27	17	32	0	0	0	0	2	4	0	0	0
0	0	0	0	0	0	167	195	165	0	0	0	11	9	10	0	0	0
0	0	1	0	0	0	320	154	199	0	0	0	42	4	12	0	0	0
0	2	0	0	0	0	275	112	101	0	0	0	13	6	5	0	0	0
2	4	2	0	0	0	100	56	66	0	0	0	12	8	12	0	0	0
7	88	36	0	0	0	169	111	154	0	0	1	25	21	21	0	0	0
0	8	9	0	0	0	56	86	137	0	0	0	8	11	14	0	0	0
1	7	8	0	0	0	43	35	48	0	0	0	3	4	5	0	0	0
2	8	13	0	0	0	19	19	21	0	0	0	4	3	6	0	0	0
0	1	1	0	0	0	4	2	3	0	0	0	0	0	0	0	0	1

human beings and cattle; C = cattle-sheds.



TABLE

Collections of adult anopheline

Year.	Month.	<i>sundaicus.</i>			<i>aconitus.</i>			<i>annularis.</i>			<i>barbirostris.</i>			<i>culicifacies.</i>			<i>hyrcanus.</i>		
		A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C
1939	May ..	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	June ..	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	July ..	0	0	0	0	0	0	3	2	7	0	0	0	0	1	2	0	0	0
	Aug. ..	0	0	0	0	0	0	44	58	57	0	0	0	40	22	27	0	0	0
	Sept. ..	0	0	0	0	0	0	55	42	39	0	0	0	21	29	33	0	0	1
	Oct. ...	0	0	0	0	0	0	19	16	14	0	0	0	17	12	22	1	1	2
	Nov. ...	0	0	0	0	0	0	22	21	31	0	0	0	2	1	3	2	2	11
	Dec. ...	0	0	0	0	0	0	8	12	37	0	0	0	0	0	0	4	6	6
1940	Jan. ...	0	0	0	0	0	0	6	18	67	1	0	0	0	0	1	0	0	2
	Feb. ...	1	1	2	0	0	0	6	15	22	0	0	1	0	0	0	0	0	0
	March ..	2	1	2	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
	April ...	2	6	8	0	0	0	0	2	1	0	0	0	0	2	0	0	0	0
	May ...	13	6	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	June ...	5	0	0	0	0	0	1	1	1	0	0	0	0	0	0	0	0	0
	July ...	0	0	1	0	0	0	2	2	42	0	0	0	1	0	4	0	0	0
	Aug. ...	3	0	0	0	0	0	1	0	9	0	0	0	3	0	1	0	0	0
	Sept. ...	9	2	3	0	0	0	2	0	12	0	1	4	2	1	1	0	0	0
	Oct. ...	12	6	13	0	0	0	4	7	25	0	0	0	1	0	0	0	3	1
	Nov. ...	180	12	28	0	0	0	3	10	46	0	0	0	0	0	0	1	0	3
	Dec. ...	8	3	3	0	0	1	0	4	24	0	0	0	0	0	0	0	0	3
1941	Jan. ...	9	5	2	0	0	0	1	5	6	0	0	0	0	0	0	0	0	2
	Feb. ...	7	10	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	March ..	2	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
	April ...	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	May ...	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	June ...	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	July ...	0	0	0	0	0	0	1	0	1	0	0	0	0	1	1	0	0	0
	Aug. ...	0	0	0	0	0	0	0	1	7	0	0	0	2	1	1	0	0	0
	Sept. ...	0	0	0	0	0	0	1	7	12	0	0	0	0	0	0	0	0	1
	Oct. ...	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Nov. ...	0	0	0	0	0	0	0	2	3	0	0	0	0	0	0	0	3	2
	Dec. ...	0	0	0	0	0	0	0	2	3	0	0	0	0	0	0	0	0	0
1942	Jan. ...	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..
	Feb. ...	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..
	March ..	0	0	2	0	0	0	0	0	3	0	0	0	0	0	0	0	0	0

A = human habitations ; B = huts occupied by both



X-g.

mosquitoes, Dubrakudi.

<i>pallidus.</i>			<i>ramsayi.</i>			<i>subpictus.</i>			<i>tessellatus.</i>			<i>vagus.</i>			<i>varuna.</i>		
A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C
0	0	0	0	0	0	2	6	8	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	2	7	7	0	0	0	0	0	0	0	0	0
0	1	0	0	0	0	193	219	253	0	0	0	3	0	2	0	0	0
0	1	2	0	0	0	474	404	573	0	0	0	11	3	10	0	0	0
2	3	2	0	0	0	298	300	356	0	0	0	1	3	1	0	0	0
3	2	4	0	0	0	74	68	76	0	0	0	1	0	3	0	0	0
1	0	3	0	0	0	50	35	50	0	0	0	2	4	8	0	0	0
1	0	2	0	0	0	44	45	85	0	0	0	0	2	8	0	0	0
0	0	1	0	0	0	27	30	104	0	0	0	0	0	0	0	0	0
0	0	1	0	0	0	11	19	45	0	0	0	0	0	1	0	0	0
0	0	0	0	0	0	15	16	50	0	0	0	0	0	1	0	0	0
0	0	0	0	0	0	30	38	72	0	0	0	0	0	2	0	0	0
0	0	0	0	0	0	79	59	66	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	182	80	123	0	0	0	2	0	0	0	0	0
0	0	0	0	0	0	255	123	342	0	0	0	4	3	7	0	0	0
0	0	0	0	0	0	230	151	531	0	0	0	2	2	30	0	0	0
0	0	0	0	0	0	132	48	255	0	0	0	48	16	83	0	0	0
0	0	0	0	0	0	166	175	223	0	0	0	11	11	19	0	0	0
0	0	0	0	0	0	124	148	515	0	0	0	1	1	0	0	0	0
0	0	0	0	0	1	145	117	336	0	0	0	0	2	5	0	0	0
0	0	0	0	0	0	136	161	200	0	0	0	6	0	10	0	0	0
0	0	0	0	0	0	100	73	200	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	147	55	137	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	20	6	43	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	17	0	5	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	18	0	30	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	112	55	132	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	169	116	255	0	0	0	3	1	4	0	0	0
0	1	1	0	0	0	92	65	125	0	0	0	2	2	0	0	0	0
0	0	0	0	0	0	3	3	8	0	0	0	0	0	0	0	0	0
0	4	3	0	0	0	20	30	59	0	0	0	3	2	8	0	0	0
0	0	0	0	0	0	14	30	39	0	0	0	3	3	2	0	0	0
..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..
0	0	0	0	0	0	5	4	6	0	0	0	0	0	0	0	0	0

human beings and cattle; C = cattle-sheds.



TABLE  
Collections of adult anopheline

Year.	Month.	sundaicus.			aconitus.			annularis.			barbirostris.			culicifacies.			hyrcanus.		
		A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C
1939	May ..	0	0	0	0	0	0	4	501	695	0	0	0	0	0	0	0	0	0
	June ..	0	0	0	0	0	0	1	955	895	0	0	0	0	0	0	0	0	0
	July ..	3	0	0	0	0	0	10	328	168	0	0	0	4	5	3	0	0	0
	Aug. ..	0	0	0	0	0	0	5	586	92	0	0	0	8	20	15	0	0	0
	Sept. ..	0	0	0	0	0	0	5	283	58	0	0	0	5	6	9	0	0	0
	Oct. ..	0	0	0	0	0	2	0	287	98	0	0	0	0	3	2	0	10	3
	Nov. ..	0	0	0	0	35	136	4	454	315	0	0	0	0	11	15	0	31	28
	Dec. ..	0	0	0	0	13	21	1	122	56	..	..	..	0	9	4	0	9	13
1940	Jan. ..	0	0	0	0	1	11	1	176	97	0	0	0	1	3	8	0	4	1
	Feb. ..	0	0	0	0	0	1	4	172	113	0	0	0	0	6	9	0	3	2
	March ..	0	0	0	0	3	2	1	303	104	0	0	0	1	6	5	0	2	3
	April ..	1	0	0	0	0	0	30	549	191	0	0	0	0	4	1	0	0	1
	May ..	0	0	0	0	0	0	30	438	141	0	0	0	0	3	0	0	0	0
	June ..	0	1	0	0	1	0	2	77	19	0	0	0	1	5	2	0	0	0
	July ..	0	2	0	0	0	0	7	298	29	0	0	0	6	62	35	0	0	0
	Aug. ..	0	0	0	0	0	0	6	138	56	0	0	0	7	25	31	0	0	0
	Sept. ..	0	0	0	0	0	1	3	192	12	0	0	0	1	30	54	0	0	1
	Oct. ..	0	0	0	0	1	1	13	219	53	0	0	0	0	2	2	0	0	1
	Nov. ..	7	11	0	0	16	1	18	464	178	0	0	0	0	0	1	0	4	0
	Dec. ..	0	0	0	16	29	88	15	451	120	..	..	..	0	2	0	0	3	5
1941	Jan. ..	5	0	0	2	21	13	10	433	254	0	0	0	0	5	0	0	4	5
	Feb. ..	4	0	0	4	25	20	11	137	127	0	0	0	0	1	1	0	3	1
	March ..	9	0	1	12	45	18	14	167	121	0	0	0	0	2	1	0	3	3
	April ..	0	0	0	3	5	4	4	101	58	0	0	0	0	1	0	0	0	0
	May ..	0	0	0	3	0	2	0	44	10	0	0	1	0	0	0	0	0	0
	June ..	7	1	0	0	0	0	29	481	201	0	0	0	0	1	0	0	0	0
	July ..	32	2	0	0	0	0	13	220	252	0	0	0	1	7	1	0	0	0
	Aug. ..	32	0	0	3	0	0	13	156	124	0	0	0	2	4	11	0	2	0
	Sept. ..	6	0	0	0	0	0	1	184	129	0	0	0	1	4	2	1	1	1
	Oct. ..	1	0	0	2	0	0	2	33	34	0	0	0	0	0	2	0	0	0
	Nov. ..	0	0	0	1	1	3	1	78	121	0	0	0	0	0	2	0	2	4
	Dec. ..	0	0	0	2	0	37	2	134	254	0	0	0	0	2	3	0	0	1
1942	Jan. ..	2	0	3	10	0	24	2	99	244	0	0	0	0	0	0	0	0	5
	Feb. ..	0	0	2	28	1	22	4	123	416	0	0	0	0	0	0	0	1	5
	March ..	0	0	1	14	0	11	6	42	152	0	0	0	0	0	0	0	0	2

A = human habitations ; B = huts occupied by both



X-h.

mosquitoes, Chatrapur.

<i>pallidus.</i>			<i>ramsayi.</i>			<i>subpictus.</i>			<i>tessellatus.</i>			<i>vagus.</i>			<i>varuna.</i>			<i>jamesi.</i>		
A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C
0	0	0	0	0	0	0	2	5	0	0	0	0	0	0	0	0	0	0	0	0
0	2	0	0	0	0	2	11	12	0	0	0	0	0	2	0	0	0	0	0	0
0	2	2	0	0	0	130	152	59	0	0	0	3	0	0	0	0	0	0	0	0
0	2	0	0	0	0	705	591	282	0	0	0	11	0	4	0	0	0	0	0	0
0	2	2	0	0	0	168	55	31	0	0	0	2	2	2	0	0	0	0	0	0
0	0	0	0	0	0	19	3	8	0	0	0	1	1	0	0	0	4	0	0	0
0	5	10	0	0	1	9	21	28	0	0	2	1	2	3	0	28	39	0	0	0
0	7	1	0	0	0	4	13	13	0	0	0	2	0	0	1	61	67	0	0	0
0	5	2	0	0	0	1	4	15	0	0	2	0	0	2	2	16	38	0	0	0
0	2	3	0	0	0	12	6	7	0	0	0	0	0	1	0	2	9	0	0	0
0	0	1	0	0	0	15	1	7	0	0	0	0	0	1	0	2	1	0	0	0
0	2	1	0	0	0	18	7	1	0	0	0	0	0	0	0	1	0	0	0	0
1	1	0	0	0	0	63	14	1	0	0	0	0	0	0	0	0	0	0	0	0
0	1	0	0	0	0	7	7	5	0	0	0	0	1	0	0	0	0	0	0	0
0	4	1	0	0	0	85	316	178	0	0	0	6	20	9	0	0	0	0	0	0
0	1	0	0	0	0	182	331	258	0	0	0	18	34	36	0	2	0	0	0	0
0	0	0	0	0	0	141	82	55	0	0	0	20	14	12	0	0	0	0	0	0
0	0	0	0	0	0	11	16	6	0	0	0	3	7	2	0	0	0	0	0	0
0	1	0	0	0	0	8	6	9	0	0	0	2	0	2	0	1	0	0	0	0
0	1	0	0	0	0	16	19	19	0	0	2	1	2	3	0	1	9	0	0	3
0	0	0	0	0	0	13	6	10	0	0	0	4	1	1	1	0	5	0	0	1
0	0	0	0	0	0	42	18	15	0	0	0	9	5	8	0	2	2	0	0	0
0	0	0	0	0	0	25	4	10	0	0	0	2	1	0	2	0	0	0	0	0
0	0	0	0	0	0	4	1	7	0	0	0	0	1	0	0	0	0	0	0	0
0	0	0	0	0	0	2	3	2	0	0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	153	35	17	0	0	0	4	0	1	0	0	0	0	0	0
1	0	0	0	0	0	233	50	55	0	0	0	15	4	4	0	0	0	0	0	0
0	0	0	0	0	0	329	114	147	0	0	0	21	6	6	1	0	0	0	0	0
0	1	1	0	0	0	798	129	153	0	0	0	38	6	6	0	0	0	0	0	0
0	3	0	0	0	0	86	22	41	0	0	0	5	1	2	0	0	0	0	0	0
0	7	12	0	0	0	12	28	57	0	0	0	2	1	10	1	0	4	0	0	0
0	1	8	0	0	0	11	14	55	0	0	2	2	2	7	1	2	21	0	0	0
0	0	1	0	0	0	0	0	18	0	0	0	0	0	2	4	0	6	0	0	0
0	0	0	0	0	0	2	3	8	0	0	0	2	1	1	0	0	3	0	0	1
0	0	0	0	0	0	2	0	4	0	0	0	0	0	0	0	0	0	0	0	0

human beings and cattle ; C = cattle-sheds.



TABLE  
Collections of adult anopheline

Year.	Month.	<i>sundaicus.</i>			<i>aconitus.</i>			<i>annularis.</i>			<i>barbirostris.</i>			<i>culicifacies.</i>		
		A	B	C	A	B	C	A	B	C	A	B	C	A	B	C
1941	January	8	..	..	..	1	..	7	14	8	..	..	..	..	1	..
	February	12	4	..	..	1	1	1	9	..	..	..	..	..	..	..
	March	1	..	..	..	..	..	4	37	28	..	..	..	..	..	..
	April	..	..	..	..	..	..	..	35	15	..	..	..	..	1	..
	May	..	..	..	..	..	..	..	18	8	..	..	..	..	..	..
	June	..	..	..	..	..	..	6	54	29	..	..	..	..	..	..
	July	..	..	..	..	..	..	12	52	25	..	..	..	1	8	1
	August	..	..	..	..	..	..	17	43	23	..	..	..	8	19	7
	September	..	..	..	..	..	..	19	47	34	..	..	..	4	17	9
	October	..	..	..	..	..	..	15	31	45	..	..	..	3	9	13
	November	..	..	..	..	..	..	83	167	198	..	..	..	..	5	4
	December	1	9	4	..	..	1	52	114	129	..	..	..	..	..	..
1942	January	2	6	2	..	4	3	33	79	101	..	..	..	..	1	..
	February	1	5	4	..	1	1	14	128	150	..	..	..	..	1	..
	March	5	4	4	..	1	..	8	57	77	..	..	..	..	1	..

A = human habitations ; B = huts occupied by both

TABLE  
Collections of adult anopheline

Year.	Month.	<i>sundaicus.</i>			<i>aconitus.</i>			<i>annularis.</i>			<i>barbirostris.</i>			<i>culicifacies.</i>		
		A	B	C	A	B	C	A	B	C	A	B	C	A	B	C
1941	January	..	..	1	..	..	..	..	..	1	..	..	..	..	..	..
	February	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..
	March	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..
	April	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..
	May	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..
	June	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..
	July	..	..	..	..	..	..	1	..	1	..	..	..	..	..	..
	August	..	..	..	..	..	..	..	..	2	..	..	..	..	..	..
	September	..	..	..	..	..	..	..	..	7	..	..	..	2	12	2
	October	..	..	..	..	..	..	..	9	37	..	..	..	..	11	7
	November	..	..	..	..	4	1	3	28	21	..	..	4	1	5	4
	December	1	1	..	2	3	..	..	13	17	..	..	..	..	..	..
1942	January	1	4	5	..	1	5	..	4	8	..	..	..	1	1	1
	February	11	6	..	2	2	2	1	4	7	..	..	..	..	3	..
	March	1	..	..	1	3	2	..	5	3	..	..	..	..	..	..

A = human habitations ; B = huts occupied by both



## X-i.

mosquitoes, *Kallikota*.

<i>hyrcanus.</i>			<i>pallidus.</i>			<i>ramsayi.</i>			<i>subpictus.</i>			<i>tessellatus.</i>			<i>vagus.</i>			<i>jamesi.</i>		
A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C
..	..	1	..	..	..	..	..	..	43	55	38	1	1	..	17	22	17	..	..	..
1	2	4	..	..	..	..	..	..	106	73	67	..	..	..	14	10	11	..	..	..
..	3	3	..	..	..	..	..	..	69	85	67	..	..	..	7	8	8	..	1	..
..	..	1	..	..	..	..	..	..	32	28	31	..	..	..	4	4	7	..	..	..
..	1	2	..	..	..	..	..	..	9	8	12	..	..	..	1	3	..	..	..	..
..	..	..	..	..	..	..	..	..	39	14	18	..	..	..	4	1	6	..	..	..
..	..	..	..	..	1	..	..	..	89	75	64	..	..	..	13	11	12	..	..	..
..	..	..	..	1	..	..	..	..	116	173	138	..	..	..	20	24	14	..	..	..
..	..	..	2	9	6	..	..	..	121	113	123	..	..	..	20	17	19	..	..	..
2	4	3	7	7	12	..	..	..	67	84	107	..	..	..	13	13	18	..	..	..
..	10	18	19	38	40	..	..	..	138	131	173	..	..	1	26	31	42	..	..	..
10	25	21	17	33	25	..	..	..	64	75	76	..	..	..	18	28	21	..	..	..
..	3	6	7	20	23	..	..	..	20	22	26	..	..	..	7	9	12	..	..	..
..	4	7	4	17	26	..	..	..	25	27	21	..	..	..	10	9	8	..	..	..
..	..	..	1	9	14	..	..	..	11	9	10	..	..	..	4	3	3	..	..	..

human beings and cattle ; C = cattle-sheds.

## X-j.

mosquitoes, *Gopalpur Area*.

<i>hyrcanus.</i>			<i>pallidus.</i>			<i>ramsayi.</i>			<i>subpictus.</i>			<i>tessellatus.</i>			<i>vagus.</i>		
A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C
..	..	..	..	..	..	..	..	..	4	..	33	..	..	..	3	4	..
..	..	..	..	..	..	..	..	..	4	..	7	1	..	5	1	..	..
..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..
..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..
..	..	..	..	..	..	..	..	..	16	23	53	1	..	2	..	..	..
..	..	1	..	..	..	..	..	..	19	10	35	..	..	..	1	..	..
..	..	..	..	1	4	..	..	1	39	76	224	..	..	..	38	66	108
..	..	1	..	..	4	..	..	6	76	183	137	..	..	..	84	140	118
2	5	9	3	7	12	..	1	3	90	188	150	..	..	1	57	72	72
6	3	8	3	3	2	..	..	..	37	55	48	1	..	..	26	22	12
..	..	2	..	2	1	..	1	..	20	36	22	1	..	1	19	22	10
1	..	..	..	..	..	..	..	..	33	44	49	..	..	..	18	9	7
..	..	..	..	..	..	..	..	..	13	11	20	..	..	..	8	13	10

human beings and cattle ; C = cattle-sheds.



TABLE

## Collections of adult anopheline

Year.	Month.	<i>sundaicus.</i>			<i>aconitus.</i>			<i>annularis.</i>			<i>barbirostris.</i>			<i>culicifacies.</i>			<i>hyrcanus..</i>		
		A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C
1941	January	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..
	February	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..
	March	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..
	April	1	..	1	..	..	..	..	..	33	..	..	..	..	..	1	..	..	..
	May	..	..	64	..	..	..	..	..	61	..	..	..	..	..	..	..	..	..
	June	..	..	..	..	..	..	2	4	50	..	..	..	..	..	..	..	..	..
	July	..	..	..	..	..	..	3	2	203	..	..	1	..	..	16	..	5	20
	August	13	4	15	..	..	..	2	90	291	..	..	..	2	8	9	..	10	75
	September	393	12	10	..	..	..	3	115	121	..	..	..	..	5	5	..	19	68
	October	157	37	28	..	..	..	3	204	373	..	..	..	..	3	5	..	32	61
	November	30	147	96	..	7	12	2	347	591	..	..	..	..	4	3	..	22	57
	December	44	81	114	..	5	10	1	108	182	..	..	..	..	2	..	..	3	28
1942	January	92	142	215	5	4	6	3	87	131	..	..	..	..	1	3	..	5	30
	February	46	55	110	4	9	2	1	89	170	..	..	..	..	2	2	..	..	23
	March	27	33	43	3	4	4	1	25	51	..	..	..	..	1	..	..	..	8

A = human habitations ; B = huts occupied by both



X-k.

mosquitoes, Ganjam Area.

<i>pallidus.</i>			<i>ramsayi.</i>			<i>subpictus.</i>			<i>tessellatus.</i>			<i>vagus.</i>			<i>varuna.</i>			<i>jamesi.</i>		
A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C
..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..
..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..
..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..
..	..	..	..	..	..	11	21	35	..	..	..	..	..	1	..	..	..	..	..	..
..	..	..	..	..	..	10	7	72	..	..	..	..	..	4	..	..	..	..	..	..
..	..	..	..	..	..	59	24	245	..	..	..	11	5	33	..	..	..	..	..	..
..	..	2	..	2	35	86	65	860	..	..	..	17	14	135	..	..	..	..	..	..
1	8	17	1	2	28	216	331	672	..	..	4	61	70	167	..	..	..	..	..	..
2	10	14	..	..	4	356	231	327	..	1	..	168	76	123	..	..	..	..	..	..
1	52	69	..	3	7	196	121	228	..	2	1	40	29	62	..	1	1	..	..	..
2	98	186	..	2	15	59	128	238	..	5	11	19	46	64	..	3	11	..	..	..
..	31	74	..	6	16	19	54	17	..	2	6	3	4	16	2	5	14	..	..	1
3	28	62	2	12	33	21	30	76	..	1	3	2	8	2	..	4	16	..	..	..
10	18	40	..	..	..	44	48	77	..	..	..	6	1	4	1	..	6	..	..	..
..	..	..	..	4	10	42	52	92	..	..	..	1	..	2	..	1	2	..	..	..

human beings and cattle ; C = cattle-sheds.



TABLE XI.  
A. *sundaicus*—Average daily catch per week.

Month.	Week.	Soran.	Sana Nairi.	Balugaon.	Kesopur.	Konoka.	Sabilla-Rambha.	Tolo Humma.	Dubrakudi.	Proyagi.	Padapodoro.	Humturi.	Komaro-Begopalli.	Chatrapur.	Haripur-Bandra.	Gopalpur.
1939	April 4	..	..	..	..	..	57	..	..	..	..	..	..	..	..	..
	1	..	..	..	..	1.0	36	..	..	..	..	..	..	0	..	..
	2	..	..	..	..	..	36	0	..	..	..	..	..	0	..	..
	3	..	..	0	..	..	6	0	0	..	..	..	..	0	..	..
May	4	..	..	0	..	1.0	0.2	0	0	..	..	..	..	0	..	..
	1	..	..	0	..	0.5	0.2	0.3	0	..	..	..	..	0	..	..
	2	..	..	0	..	0	0	0	0	..	..	..	..	0	..	..
	3	..	0	0	0	0	0.6	0	0	..	..	..	..	0	..	..
June	4	..	0	0	0	0	0	0	0	..	..	..	..	0	..	..
	5	..	0	0	0	0	0	0	0	..	..	..	..	0	..	..
	1	..	0	0	0	0	0	0	0	..	..	..	..	1.0	..	..
	2	..	0	0	0	0	0	0	0	..	..	..	..	0	..	..
July	3	..	0	0	0	0	0	0	0	..	..	..	..	0	..	..
	4	..	0	0	0	0	0	0	0	..	..	..	..	0	..	..







TABLE XI—*contd.*

Month.	Week.	Goran.	Sana Nairi.	Balugaon.	Kesopur.	Konoka.	Sablia-Rambha.	Tolo Humma.	Dubrakudi.	Proyagi.	Padapodoro.	Hummitri.	Komaro-Begopalli.	Chattrapur.	Haripur-Bandra.	Gopalpur.
1940	1	..	0	0	0	0	0	0	0	..	..	..	..	0	..	0
	2	..	0	0	0	0	0	0	0	..	..	..	..	0	..	..
	3	..	0.3	0	0	0	0	1.0	0	..	0	0	0	0	..	..
	4	..	0	0	0	0	1.0	0.6	0	..	..	..	..	0	..	..
January	5	..	0	0	0	0	0.4	0	0	..	..	..	..	0	1.5	0
	1	0	0	0	0	0	0.3	0	0	0	..	..	..	0	..	..
	2	..	0	0	0	0	0.3	0	0	..	..	..	..	0	0	0
	3	..	0	0	0	0.3	0.4	0	0.5	..	0	0	0	0	..	..
February	4	..	0	0	0	0.7	11.1	0	1.2	..	..	..	..	0	..	..
	1	..	0	0	0	1.7	21.0	0	0.3	1.0	..	..	..	0	..	..
	2	..	0	0	1.0	0.3	5.4	0	0	..	0	0	0	0	..	..
	3	..	0.5	0	0	1.7	16.7	2.0	0.6	..	..	..	..	0	..	..
March	4	..	0.7	0	0	1.0	11.5	0	0.6	..	..	..	..	0	..	..
	1	0	0	0.3	0	2.4	14.2	1.7	1.6	1.0	..	..	..	0	..	..
	2	..	0	0	0.5	3.7	62.4	1.5	2.0	..	..	..	..	0	..	..
	3	..	0.5	0	0	0.3	31.6	1.5	0.0	..	0	0	0	0	..	..
April	4	..	0	0	0	1.3	33.6	0.3	1.0	..	..	..	..	0.3	..	0







TABLE XI—*contd.*

Month.	Week.	Soran.	Sana Nairi.	Balugaon.	Kesapur.	Konoka.	Sabilla-Rambha.	Tolo Humma.	Dubrakudi.	Proyagi.	Padapodoro.	Hummiti.	Komaro-Begopalli.	Chattrapur.	Haripur-Bandra.	Gopalpur.
1940 October	1	0	0.3	0	95.5	3.5	1.0	4.0	0.0	..	..	..	..	0	..	..
	2	..	..	0	105.6	3.0	1.0	5.0	1.0	30.0	..	..	..	0	..	..
	3	0	3.0	0	137.8	4.0	5.4	5.0	3.0	..	0	0	0	0	..	..
	4	..	0.7	0	112.7	1.3	6.3	9.3	8.5	139.0	..	..	..	0	..	..
	5	0	6.0	0	92.0	7.5	6.0	3.0	..	132.0	..	..	..	0	..	..
November	1	0	4.3	0	134.3	3.0	5.3	0.5	51.3	139.0	..	..	..	0	..	..
	2	0	6.5	0	83.0	6.3	8.9	3.3	15.3	..	0	0	0	6.9	..	..
	3	..	0.3	0	57.3	13.5	6.0	4.6	3.7	..	..	..	..	0.0	..	..
	4	..	8.4	..	42.0	30.3	1.3	2.4	4.5	..	..	..	..	0.5	..	..
December	1	..	7.0	..	72.7	8.5	0.5	1.9	2.0	..	..	..	..	0	..	..
	2	..	6.4	..	127.5	2.0	1.0	3.7	0.7	..	..	..	..	0	..	..
	3	..	3.3	..	56.3	16.3	4.4	3.7	1.0	..	..	..	..	0	..	..
	4	0	0	..	44.0	..	2.0	..	3.0	..	..	..	..	0	..	..



[illegible]



**TABLE XI—*contd.***

Month.	Week.	Goran.	Sana Nairi.	Balugaon.	Kesopur.	Konoka.	Sabilla-Rambha.	Tolo Humma.	Dubrakudi.	Proyagi.	Padaipodoro.	Hummiri.	Komaro-Begopalli.	Chattrapur.	Harpur-Bandra.	Gopalpur.
1941	1	..	0	0	0	:	0	0	0	0	:	:	:	0	:	:
	2	..	0	0	0	0	0	0	:	:	:	:	:	0	:	:
	3	0	0	0	0	:	0	0	0	:	:	:	:	0	:	:
	4	..	0	0	0	0	0	0	0	:	0	0	0	1	:	:
June	1	0	0	0	0	0	0	0	0	:	:	:	:	4	:	:
	2	0	0	0	0	0	0	0	0	:	:	:	:	3	:	:
	3	0	0	0	0	0	0	0	0	0	:	:	:	3	:	:
	4	0	0	0	0	0	0	0	0	:	0	0	0	6	:	:
	5	0	0	0	0	0	0	0	0	:	:	:	:	4	2	0
July	1	0	0	0	0	0	0	0	0	0	:	:	:	17	:	:
	2	0	0	0	0	0	0	0	0	:	:	:	:	10	:	:
	3	0	0	0	0	0	0	0	0	:	0	0	0	1	:	:
	4	0	0	0	0	0	0	0	0	:	0	0	0	1	:	:
	5	0	0	0	0	0	0	0	0	:	:	:	:	2	:	0
August	1	0	0	0	0	0	0	0	0	:	:	:	:	17	:	:
	2	0	0	0	0	0	0	0	0	:	:	:	:	10	:	:
	3	0	0	0	0	0	0	0	0	:	0	0	0	1	:	:
	4	0	0	0	0	0	0	0	0	:	0	0	0	2	:	0



[illegible]



**TABLE XI—concl'd.**

Month.	Week.	Soran.	Sana Nairi.	Balugaon.	Kesopur.	Konoka.	Sablia-Kambha.	Tolo Humma.	Dubrakudi.	Proyagi.	Pada-podoro.	Hummiri.	Komaro-Begopalli.	Chattrapur.	Haripur-Bandra.	Gopalpur.
1942 February ..	1	..	113-0	1-0	3-5	0	0	0	:	:	:	:	:	1-0	0	2-0
	2	..	..	0	6-0	0	0	0	:	:	:	:	:	0	0	2-5
	3	..	56-0	0	2-0	0	0	0	:	:	:	:	:	0-5	0-5	2-5
	4	..	..	0-2	2-0	0	0	0-3	:	:	:	:	:	0	0	1-5
March ..	1	..	90-0	0	3-0	0	0	0	2-0	:	:	:	:	0	0	1-0
	2	..	64-0	0	11-0	0	0	0	:	1-0	:	:	:	..	0	0
	3	..	118-0	0	8-5	0	0	0	:	:	2-0	:	0	0-5	0	0
	4	..	68-0	..	..	..	..	..	:	:	:	:	:	..	..	..



TABLE XII.

Locality.	Date.	Type of breeding place.	Salinity parts per 100,000.	Species found breeding.	Number of each species.	Type of aquatic vegetation.
	1941					
Soran	Nov. ..	Chilka Lake	130	<i>sundaicus</i>	10	Not recorded.
				<i>subpictus</i>	3	
"	Dec. ..	Do.	125	<i>sundaicus</i>	16	Heavy growth of Potamogeton, Najas, Oscillatoria, Lyngbya.
				<i>subpictus</i>	6	
				<i>annularis</i>	1	
	1940					
Sana Nairi	May ..	Well (9 ft. below ground level).	50	<i>sundaicus</i>	1	No weeds.
				<i>subpictus</i>	11	
				<i>barbirostris</i>	1	
"	Sept. ..	Tank 42B	40	<i>sundaicus</i>	5	Lemna, Cladophora, Oedogonium.
				<i>culicifacies</i>	16	
				<i>subpictus</i>	2	
				<i>vagus</i>	4	
				<i>hyrcanus</i>	5	
				<i>varuna</i>	5	
"	Oct. ..	Do.	230	<i>sundaicus</i>	21	Do.
				<i>hyrcanus</i>	1	
				<i>subpictus</i>	2	
				<i>vagus</i>	6	
"	Nov. ..	Do.	250	<i>sundaicus</i>	23	Do.
"	Dec. ..	Do.	300	<i>sundaicus</i>	1	Lemna only.
"	Dec. ..	Chilka Lake 42	375-500	<i>sundaicus</i>	7	Putrefying alga—Anabaena, Chaetomorpha, Diatoms and Desmids, Potamogeton and Chara.
				<i>subpictus</i>	116	
				<i>annularis</i>	2	



TABLE XII—*contd.*

Locality.	Date.	Type of breeding place.	Salinity parts per 100,000.	Species found breeding.	Number of each species.	Type of aquatic vegetation.
Sana Nairi	1941	Chilka Lake 42	525-600	<i>sundaicus</i>	24	Same as above, but putrefying algae less abundant.
	Jan. ..			<i>subpictus</i>	58	
				<i>annularis</i>	1	
	Feb. ..	Do.	625-800	<i>sundaicus</i>	320	Do., but putrefying algae increasing.
				<i>subpictus</i>	70	
	March ..	Do.	850	<i>sundaicus</i>	82	Potomogeton, Spirogyra, Oscillatoria, Chaetomorpha.
				<i>subpictus</i>	227	
	April ..	Do.	850-950	<i>sundaicus</i>	24	Do., but less abundant.
				<i>subpictus</i>	160	
	Oct. ..	Do.	350	<i>sundaicus</i>	1	Heavy growth of Potomogeton, Najas, Lyngbya, Cladophora, Oscillatoria, Spirogyra.
				<i>subpictus</i>	14	
	Nov. ..	Do.	250	<i>sundaicus</i>	18	Do.
				<i>subpictus</i>	48	
	Dec. ..	Do.	275	<i>sundaicus</i>	23	Do., very heavy and extensive growth.
				<i>subpictus</i>	56	
1942	Jan. ..	Do.	525	<i>sundaicus</i>	12	Do.
<i>subpictus</i>				19		
Feb. ..	Do.	575	<i>sundaicus</i>	14	Do., but weeds beginning to die.	
			<i>subpictus</i>	14		
March	Do.	725	<i>sundaicus</i>	18	Do.	
			<i>subpictus</i>	14		
1941	April ..	Tank 41	25	<i>sundaicus</i>	3	Ceratophyllum, Hydrilla, Nymphaea, Lyngbya, scanty Oscillatoria.
<i>subpictus</i>				1		
<i>annularis</i>				4		



TABLE XII—*contd.*

Locality.	Date.	Type of breeding place.	Salinity parts per 100,000.	Species found breeding.	Number of each species.	Type of aquatic vegetation.
Sana Nairi	1941					
	May ..	Tank 41	25	<i>sundaicus</i>	1	Ceratophyllum, Hydrilla, Nymphae, scanty Lyngbya and Spirogyra.
				<i>subpictus</i>	7	
				<i>annularis</i>	2	
	March ..	Nala 38, near shore of Lake.	925	<i>sundaicus</i>	6	Spirogyra, Oscillatoria.
	Dec. ..	Do.	15	<i>sundaicus</i>	2	Floating putrefying plaques of algæ in continuation with Chilka Lake weed belts.
				<i>annularis</i>	23	
"	1942					
	Jan. ..	Nala 38	500	<i>sundaicus</i>	12	Do.
				<i>subpictus</i>	9	
	Feb. ..	Do.	625	<i>sundaicus</i>	3	Do.
				<i>subpictus</i>	3	
	March ..	Do.	..	<i>sundaicus</i>	10	Do.
				<i>subpictus</i>	7	
"	1941					
	April ..	Chilka Lake (disused boat).	900	<i>sundaicus</i>	1	Spirogyra.
				<i>subpictus</i>	7	
	1942					
	Jan. ..	Tank 42C	15	<i>sundaicus</i>	4	Najas.
				<i>annularis</i>	6	
Balugaon	1941					
	March ..	Chilka Lake 54	800-875	<i>sundaicus</i>	6	Potomogeton, Chætormorpha, Spirogyra, Lyngbya.
				<i>subpictus</i>	71	
	April ..	Do.	875-900	<i>sundaicus</i>	2	Potomogeton, Spirogyra, Enteromorpha, Lyngbya, Oscillatoria, all scanty.
				<i>subpictus</i>	42	



TABLE XII—*contd.*

Locality.	Date.	Type of breeding place.	Salinity parts per 100,000.	Species found breeding.	Number of each species.	Type of aquatic vegetation.
Balugaon	1941					
	Dec. ..	Chilka Lake 54	525	<i>sundaicus</i>	1	Very scanty marginal growth of algæ and Potamogeton.
	"	"	"	<i>subpictus</i>	28	
	March ..	Tank 46	25	<i>sundaicus</i>	4	Ceratophyllum, Najas, Nympha, Cladophora and Spirogyra.
	"	"	"	<i>annularis</i>	47	
	"	"	"	<i>subpictus</i>	4	
	Nov. ..	Pool near shore	140	<i>sundaicus</i>	4	Marginal growth of Lyngbya and Spirogyra.
	"	"	"	<i>subpictus</i>	15	
Kesopur	Dec. ..	Do.	625	<i>sundaicus</i>	2	Do.
	"	"	"	<i>subpictus</i>	39	
	1940					
	Aug. ..	Ricefield flooded by lake water.	275	<i>sundaicus</i>	28	Putrefying algæ, Lyngbya and Spirogyra.
	"	"	"	<i>subpictus</i>	84	
	"	"	"	<i>vagus</i>	12	
	Sept. ..	Do.	700	<i>sundaicus</i>	13	Do.
	"	"	"	<i>subpictus</i>	420	
	"	"	"	<i>vagus</i>	100	
	Sept. ..	Swamp 3 (originally connected with lake).	740	<i>sundaicus</i>	72	<i>Lyngbya æstuaris</i> , <i>L. confervoides</i> , <i>Anabaena</i> , <i>Najas</i> (Lyngbya abundant).
	"	"	"	<i>subpictus</i>	142	
	"	"	"	<i>vagus</i>	15	
	Oct. ..	Do.	670	<i>sundaicus</i>	39	Do.
	"	"	"	<i>subpictus</i>	85	
	"	"	"	<i>vagus</i>	16	
	Nov. ..	Do.	770	<i>sundaicus</i>	28	Lyngbya, Anabaena, Protococcus, Najas. Putrefying algæ decreasing.
	"	"	"	<i>subpictus</i>	121	
	"	"	"	<i>vagus</i>	11	



TABLE XII—*contd.*

Locality.	Date.	Type of breeding place.	Salinity parts per 100,000.	Species found breeding.	Number of each species.	Type of aquatic vegetation.
Kesopur	1940	Swamp 3 (originally connected with lake).	725	<i>sundaicus</i>	14	Further marked decrease in putrefying algæ.
	<i>subpictus</i>			73		
	<i>vagus</i>			8		
"	1941	Do.	700-750	<i>sundaicus</i>	10	Do.
	<i>subpictus</i>			80		
	<i>vagus</i>			1		
"	Feb. ..	Do.	750-900	<i>sundaicus</i>	7	Swamp drying up. Pools in bed containing scanty putrefying algæ.
	<i>subpictus</i>	56				
"	March ..	Do.	900-1,000	<i>sundaicus</i>	8	Scanty growth of <i>Lyngbya</i> , <i>Oscillatoria</i> , <i>Najas</i> .
	<i>subpictus</i> (no breeding after 10.iii).	101				
"	Sept. ..	Swamp 5 (originally connected with lake).	595	<i>sundaicus</i>	35	<i>Lyngbya æstuarii</i> , <i>L. ærugineo-coerulea</i> , <i>L. confervoides</i> , <i>Anabæna</i> , <i>Najas</i> . ( <i>Lyngbya</i> abundant.)
	<i>subpictus</i>			85		
	<i>vagus</i>			10		
"	Oct. ..	Do.	640	<i>sundaicus</i>	30	Do.
	<i>subpictus</i>	105				
	<i>vagus</i>	15				
"	Nov. ..	Do.	700	<i>sundaicus</i>	63	Do.
	<i>subpictus</i>	79				
	<i>vagus</i>	18				
"	Dec. ..	Do.	650	<i>sundaicus</i>	24	<i>Lyngbya</i> spp., <i>Oscillatoria</i> , <i>Chlorella</i> , <i>Anabæna</i> , <i>Najas</i> .
	<i>subpictus</i>	30				
	<i>vagus</i>	6				
"	Oct. ..	Swamp 4A (subject to lake flooding).	700	<i>sundaicus</i>	15	<i>Lyngbya æstuarii</i> , <i>L. ærugineo-coerulea</i> , <i>L. confervoides</i> , <i>Anabæna</i> , <i>Najas</i> .
	<i>subpictus</i>			28		



TABLE XII—*contd.*

Locality.	Date.	Type of breeding place.	Salinity parts per 100,000.	Species found breeding.	Number of each species.	Type of aquatic vegetation.
Kesopur	1941					
	Nov. ..	Swamp 4A (subject to lake flooding).	895	<i>sundaicus</i>	33	<i>Lyngbya æstuarii</i> , <i>L. ærugineo-coerulea</i> , <i>L. confervoides</i> , <i>Anabæna</i> , <i>Najas</i> .
				<i>subpictus</i>	127	
				<i>vagus</i>	15	
	Dec. ..	Do.	942	<i>sundaicus</i>	10	<i>Lyngbya</i> , <i>Oscillatoria</i> , <i>Chlorella</i> , <i>Anabæna</i> , <i>Xenococcus</i> , <i>Najas</i> .
				<i>subpictus</i>	62	
				<i>vagus</i>	5	
	1942					
	Jan. ..	Do.	1,000–1,250	<i>sundaicus</i>	3	Do.
				<i>subpictus</i>	7	
	1940					
	Oct. ..	Swamp 4B	600	<i>sundaicus</i>	21	<i>L. æstuarii</i> , <i>L. ærugineo-coerulea</i> and other algæ.
				<i>subpictus</i>	28	
	Nov. ..	Do.	735	<i>sundaicus</i>	36	Do.
				<i>subpictus</i>	116	
				<i>vagus</i>	18	
	Dec. ..	Do.	..	<i>sundaicus</i>	18	Do.
				<i>subpictus</i>	37	
				<i>vagus</i>	5	
	1941					
	Jan. ..	Swamp 4B	625–650	<i>sundaicus</i>	10	<i>Najas</i> , <i>Lyngbya</i> , <i>Anabæna</i> .
				<i>subpictus</i>	43	
	Feb. ..	Do.	925–2,250	<i>sundaicus</i>	4	Algæ almost absent. Scanty growth of <i>Najas</i> .
				<i>subpictus</i>	40	
	1940					
	Nov. ..	Swamp 6B	869	<i>sundaicus</i>	83	<i>Lyngbya</i> , <i>Oscillatoria</i> , <i>Chætomorpha</i> , <i>Anabæna</i> , <i>Spirogyra</i> , <i>Cylindrospermum</i> .
				<i>subpictus</i>	102	
				<i>vagus</i>	20	



TABLE XII—*contd.*

Locality.	Date.	Type of breeding place.	Salinity parts per 100,000.	Species found breeding.	Number of each species.	Type of aquatic vegetation.
Kesopur	1940	Swamp 6B	733	<i>sundaicus</i>	48	Lyngbya, Oscillatoria, Chætomorpha, Anabæna, Spirogyra, Cyndrospermum.
	Dec. ..			<i>subpictus</i>	32	
				<i>vagus</i>	5	
	1941	Do.	775-850	<i>sundaicus</i>	25	Putrefying algæ; Lyngbya, Oscillatoria, Chætomorpha, Anabæna, Spirogyra.
	Jan. ..			<i>subpictus</i>	80	
				<i>vagus</i>	2	
	Feb. ..	Do.	Swamp dried on 10.ii.41.	<i>sundaicus</i>	3	Do., but scanty.
				<i>subpictus</i>	9	
	1940	Tank 6	850	<i>sundaicus</i>	5	Putrefying Lyngbya, Nymphæacæ, Najas.
	Aug. ..			<i>subpictus</i>	27	
	Sept. ..	Do.	483	<i>sundaicus</i>	14	Lyngbya æstuarii, L. ærugineo-coerulea, L. confervoides, Najas, Nymphæacæ.
				<i>subpictus</i>	83	
				<i>vagus</i>	13	
	Oct. ..	Do.	680	<i>sundaicus</i>	57	Do.
				<i>subpictus</i>	87	
				<i>vagus</i>	10	
	Nov. ..	Do.	775	<i>sundaicus</i>	91	Do.
				<i>subpictus</i>	91	
				<i>vagus</i>	17	
				<i>aconitus</i>	3	
	Dec. ..	Do.	750	<i>sundaicus</i>	71	Lyngbya spp., Oscillatoria spp., Chlorella, Najas.
				<i>subpictus</i>	19	
				<i>vagus</i>	8	
				<i>aconitus</i>	2	



TABLE XII—contd.

Locality.	Date.	Type of breeding place.	Salinity parts per 100,000.	Species found breeding.	Number of each species.	Type of aquatic vegetation.
Kesopur	1941					
	Jan. ..	Tank 6	600-850	<i>sundaicus</i>	50	Putrefying algæ; Lyngbya, Oscillatoria, Chlorella, Spirogyra, Najas.
				<i>subpictus</i>	64	
	Feb. ..	Do.	925-1,000	<i>sundaicus</i>	20	Putrefying algæ; Lyngbya, Oscillatoria, Chlorella, Spirogyra, Najas; tank drying.
				<i>subpictus</i>	50	
	March ..	Do.	1,000-1,700	No breeding	..	Water receding, free from weeds on 14.iii.41.
	April ..	Do.	2,500	Do.	..	Small pool left. No weeds.
	Nov. ..	Do.	775	<i>sundaicus</i>	15	Najas, floating algæ; Lyngbya, Oscillatoria, Spirogyra.
				<i>subpictus</i>	507	
				<i>vagus</i>	42	
	Dec. ..	Do.	825	<i>sundaicus</i>	41	Do.
				<i>subpictus</i>	514	
				<i>vagus</i>	17	
"	1942					
	Jan. ..	Do.	1,300	<i>sundaicus</i>	15	Do.
				<i>subpictus</i>	168	
				<i>vagus</i>	7	
	Feb. ..	Do.	1,150-1,650	<i>sundaicus</i>	2	Very scanty marginal growth of algæ. Free from weeds on 12.ii.
				<i>subpictus</i> (up to 7.ii)	24	
				<i>subpictus</i> (on 12.ii)		
				No breeding from 16.ii.		
	March ..	Chilka Lake 4	850	<i>sundaicus</i>	6	Scanty growth of Lyngbya, Oscillatoria, Najas.
				<i>subpictus</i>	89	
"	Feb. ..	Borrowpit 7B	625	<i>sundaicus</i>	9	Najas, Lyngbya, Spirogyra.
				<i>subpictus</i>	32	
	March ..	Do.	1,125	<i>sundaicus</i>	4	
				<i>subpictus</i>	40	..



TABLE XII—contd.

Locality.	Date.	Type of breeding place.	Salinity parts per 100,000.	Species found breeding.	Number of each species.	Type of aquatic vegetation.
Kesopur	1942	Tank 1	30	<i>sundaicus</i>	23	Hydrilla, Najas. Scanty growth of Ceratophyllum, Lyngbya, Cladophora.
	March ..			<i>subpictus</i>	38	
				<i>annularis</i>	33	
Kallikota	1941	Nuagai Tank	25	<i>sundaicus</i>	1	..
	Feb. ..			<i>subpictus</i>	30	
,,	1942	Do.	20	<i>sundaicus</i>	7	Najas, Hydrilla, Cladophora, Spirogyra, Anabaena.
	Jan. ..			<i>annularis</i>	91	
				<i>subpictus</i>	9	
,,	Feb. ..	Do.	20	<i>sundaicus</i>	4	Do.
				<i>annularis</i>	58	
				<i>subpictus</i>	7	
,,	Feb. ..	Lingi Tank	25	<i>sundaicus</i>	13	Hydrilla, Najas, putrefying Cladophora and other algae.
				<i>subpictus</i>	4	
				<i>annularis</i>	54	
				<i>pallidus</i>	6	
,,	March ..	Do.	35	<i>sundaicus</i>	14	Do.
				<i>subpictus</i>	13	
				<i>annularis</i>	54	
				<i>pallidus</i>	12	
Konoka	1940	Borrowpit 5	800	<i>sundaicus</i>	4	Putrefying algae; Lyngbya spp., Najas.
	April ..			<i>subpictus</i>	22	
,,	April ..	Borrowpit 8	900	<i>sundaicus</i>	9	Do.
				<i>subpictus</i>	13	



TABLE XII—contd.

Locality.	Date.	Type of breeding place.	Salinity parts per 100,000.	Species found breeding.	Number of each species.	Type of aquatic vegetation.
Konoka	1940 Sept. ..	Swamp, near shore of lake.	1,100	<i>sundaicus</i>	1	Putrefying algæ; Lyngbya spp., Anabæna, Najas.
	<i>subpictus</i>			22		
	<i>vagus</i>			2		
"	Oct.	Do.	542-800	<i>sundaicus</i>	12	Do.
	<i>subpictus</i>	153				
	<i>vagus</i>	10				
"	Nov. ..	Do.	700-825	<i>sundaicus</i>	29	Do.
	<i>subpictus</i>	82				
	<i>vagus</i>	9				
"	Dec. ...	Do.	775-875	<i>sundaicus</i>	12	Do.
	<i>subpictus</i>	47				
	<i>vagus</i>	4				
"	1941 Jan. ..	Do.	750-850	<i>sundaicus</i>	17	Putrefying algæ; Lyngbya, scanty Anabæna, Najas.
	<i>subpictus</i>			181		
	"			Feb. ..	Do.	
<i>subpictus</i>		96				
"		1940 Sept. ..	Ricefield 5A	750	<i>sundaicus</i>	1
	<i>subpictus</i>	27				
	<i>vagus</i>	2				
"	Oct. ..	Do.	1,200	<i>sundaicus</i>	15	Do.
	<i>subpictus</i>	140				
	<i>vagus</i>	20				
"	1941 Dec. ..	Swamp, near shore of lake.	800	<i>sundaicus</i>	2	Putrefying algæ.
	<i>subpictus</i>			14		



TABLE XII—*contd.*

Locality.	Date.	Type of breeding place.	Salinity parts per 100,000.	Species found breeding.	Number of each species.	Type of aquatic vegetation.
Konoka	1942	Borrowpit 6	..	<i>sundaicus</i>	1	No vegetation.
	Jan. ..			<i>subpictus</i>	8	
	1939					
Sabilia	April ..	Khuntia Tank 3	700	<i>sundaicus</i>	..	Putrefying algæ.
				<i>subpictus</i>	..	
"	May ..	Do.	800	<i>sundaicus</i>	15	Do.
				<i>subpictus</i>	24	
"	June ..	Do.	900	<i>sundaicus</i>	1	Putrefying algæ scanty; Najas.
				<i>subpictus</i>	24	
"	1940	Do.	400	<i>sundaicus</i>	3	Scanty putrefying Lyngbya, Potomogeton, Najas.
	March ..			<i>subpictus</i>	97	
"	April ..	Do.	525	<i>sundaicus</i>	1	Do., no Potomogeton.
				<i>subpictus</i>	27	
"	1941	Do.	300	<i>sundaicus</i>	22	Heavy growth of Najas, scanty growth of Lyngbya, Spirogyra.
	Jan. ..			<i>subpictus</i>	45	
				<i>annularis</i>	137	
"	Feb. ..	Do.	350	<i>sundaicus</i>	59	Do., heavy growth. Cleaned on 8-10.ii. No breeding.
				<i>subpictus</i>	100	
				<i>annularis</i> (up to 10.ii)	66	
"	1940	Pucha Tank 2A	725	<i>sundaicus</i>	90	Putrefying floating Lyngbya.
	March ..			<i>subpictus</i>	85	
"	April ..	Do.	825-975	<i>sundaicus</i>	191	Do.
				<i>subpictus</i>	341	



TABLE XII—*contd.*

Locality.	Date.	Type of breeding place.	Salinity parts per 100,000.	Species found breeding.	Number of each species.	Type of aquatic vegetation.
Sabilia	1940					
	May ..	Pucha Tank 2A	300-1,025	<i>sundaicus</i>	5	Putrefying floating Lyngbya during first half of month. Filled up with rainwater in the third week.
				<i>subpictus</i>	42	
	June ..	Do.	400	<i>sundaicus</i>	3	Scanty marginal growth of Lyngbya.
				<i>subpictus</i>	68	
	Oct. ..	Do.	500	<i>sundaicus</i>	7	Marginal growth of Lyngbya up to 20.x.
				<i>subpictus</i>	29	
	Nov. ..	Do.	350	<i>sundaicus</i>	6	Putrefying Lyngbya, Chætomorpha.
				<i>subpictus</i>	21	
				<i>vagus</i>	1	
	Dec. ..	Do.	375	<i>sundaicus</i>	3	Do.
				<i>subpictus</i>	13	
"	1941					
	Feb.	Do.	525	<i>sundaicus</i>	29	Putrefying Chætomorpha.
				<i>subpictus</i>	82	
	March ..	Do.	600-850	<i>sundaicus</i>	13	Do.
				<i>subpictus</i>	106	
	1939					
"	May ..	Tamna Tank 19B.	100	<i>sundaicus</i>	7	Pistia, Ceratophyllum.
				<i>annularis</i>	1	
				<i>pallidus</i>	1	
				<i>subpictus</i>	27	
"	1941					
	April ..	Do.	400	<i>sundaicus</i>	21	Do.
				<i>annularis</i>	67	
				<i>hyrcanus</i>	3	



TABLE XII—contd.

Locality.	Date.	Type of breeding place.	Salinity parts per 100,000.	Species found breeding.	Number of each species.	Type of aquatic vegetation.
Sabilia	1939					
	May ..	Nista Palli Tank 6.	45	<i>sundaicus</i>	12	Hydrilla, Nymphaea.
				<i>subpictus</i>	18	
"				<i>annularis</i>	4	
	June ..	Do.	50	<i>sundaicus</i>	1	Do.
				<i>subpictus</i>	13	
"	1941					
	March ..	Do.	425	<i>sundaicus</i>	6	Hydrilla, Ceratophyllum.
				<i>annularis</i>	101	
"				<i>subpictus</i>	5	
"	1940					
	May ..	Kundra Tank (Rambha).	450	<i>sundaicus</i>	2	Hydrilla, Najas.
				<i>subpictus</i>	400	
"	May ..	Midra Tank (Rambha).	1,100	<i>sundaicus</i>	4	Putrefying floating algae; Lyngbya spp.
				<i>subpictus</i>	260	
"	1939					
	June ..	Kacha temp. well near lake.	600	<i>sundaicus</i>	1	No weeds.
				<i>subpictus</i>	9	
"	June ..	Well in Kela Garden (18 ft. below ground level).	150	<i>sundaicus</i>	1	Do.
"	1940					
	Nov. ..	Chilka Lake 1	850	<i>sundaicus</i>	1	Putrefying Lyngbya, Spirogyra, Oscillatoria, Najas, Potamogeton.
				<i>subpictus</i>	470	
				<i>vagus</i>	48	



TABLE XII—contd.

Locality.	Date.	Type of breeding place.	Salinity parts per 100,000.	Species found breeding.	Number of each species.	Type of aquatic vegetation.
Sabilia	1941					
	Jan. ..	Chilka Lake 1	650	<i>sundaicus</i>	2	Putrefying plaques of algæ mostly Lyngbya, Najas.
				<i>subpictus</i>	339	
	March ..	Do.	875	<i>sundaicus</i>	4	Heavy growth of Potomogeton, Najas, Lyngbya; scanty growth of Oscillatoria, Enteromorpha, Gracillaria.
				<i>subpictus</i>	507	
	April ..	Do.	875	<i>sundaicus</i>	2	Do., but less abundant.
				<i>subpictus</i>	110	
	Jan. ..	Nala 24E	525	<i>sundaicus</i>	1	Putrefying algæ; Lyngbya, Oscillatoria, Najas.
				<i>subpictus</i>	4	
	Jan. ..	Nala 26	15	<i>sundaicus</i>	3	Ceratophyllum, Najas, Hydrilla, Nymphaea, Cladophora, Spirogyra.
				<i>annularis</i>	34	
				<i>jamesi</i>	5	
	Jan. ..	Tank 19A	300	<i>sundaicus</i>	1	Pistia, Spirogyra, very scanty.
				<i>annularis</i>	30	
				<i>hyrcanus</i>	3	
				<i>jamesi</i>	1	
	Jan. ..	Borrowpit 2F, 2F.	900	<i>sundaicus</i>	3	Masses of algæ.
				<i>subpictus</i>	9	
	Feb. ..	Do.	1,125	<i>sundaicus</i>	8	Do.
				<i>subpictus</i>	32	
	Jan. ..	Borrowpit 40	375	<i>sundaicus</i>	1	No weeds.
				<i>annularis</i>	3	
				<i>vagus</i>	3	
				<i>barbirostris</i>	1	
	March ..	Borrowpit 41	1,300	<i>sundaicus</i>	7	Putrefying algæ; Lyngbya, Oscillatoria.
				<i>subpictus</i>	196	



TABLE XII—contd.

Locality.	Date.	Type of breeding place.	Salinity parts per 100,000.	Species found breeding.	Number of each species.	Type of aquatic vegetation.
Tolo Humma	1940					
	July ..	Well 41 (Ground level).	135	<i>sundaicus</i>	2	No weeds.
				<i>culicifacies</i>	30	
				<i>subpictus</i>	120	
	Oct. ..	Do. (2½ ft. below ground level).	300	<i>sundaicus</i>	2	Do.
				<i>subpictus</i>	14	
	Nov. ..	Do. (3 ft. below ground level).	450	<i>sundaicus</i>	3	Do.
				<i>subpictus</i>	9	
	Jan. ..	Do. (5 ft. below ground level).	500	<i>sundaicus</i>	3	Do.
				<i>subpictus</i>	14	
				<i>varuna</i>	1	
				<i>vagus</i>	2	Do.
	Feb. ..	Do. (6 ft. below ground level).	500	<i>sundaicus</i>	15	
				<i>subpictus</i>	106	
				<i>vagus</i>	4	
				<i>varuna</i>	29	Scanty floating <i>Lyngbya</i> , <i>Spirogyra</i> .
	Oct. ..	Moat Channel 46.	525	<i>sundaicus</i>	5	
				<i>subpictus</i>	14	Do.
	Nov. ..	Do.	775	<i>sundaicus</i>	3	
				<i>subpictus</i>	17	Do.
	Dec. ..	Do.	713	<i>sundaicus</i>	16	
				<i>subpictus</i>	14	
				<i>vagus</i>	2	Scanty putrefying algae; <i>Lyngbya</i> , <i>Spirogyra</i> .
	1941					
	Jan. ..	Do.	800- 1,400	<i>sundaicus</i>	6	
				<i>subpictus</i>	46	



TABLE XII—contd.

Locality.	Date.	Type of breeding place.	Salinity parts per 100,000.	Species found breeding.	Number of each species.	Type of aquatic vegetation.
Tolo Humma	1941					
	Dec. ..	Moat Channel 46.	300-425	<i>sundaicus</i>	7	Scanty marginal floating algæ; Lyngbya, Spirogyra.
				<i>subpictus</i>	45	
	Feb. ..	Tank 3	25	<i>sundaicus</i>	6	Hydrilla, Ceratophyllum, scanty growth of Spirogyra and Oscillatoria.
				<i>subpictus</i>	14	
				<i>annularis</i>	12	
				<i>hyrcanus</i>	6	
				<i>jameoi</i>	3	
	Feb. ..	Borrowpit 5	20	<i>sundaicus</i>	1	Ceratophyllum, Spirogyra.
				<i>annularis</i>	70	
..	March ..	Do.	25	<i>sundaicus</i>	3	Do.
				<i>subpictus</i>	11	
				<i>vagus</i>	7	
	March ..	Tank 20	45	<i>sundaicus</i>	2	Hydrilla, Ceratophyllum, Lemna, heavy growth of Spirogyra.
				<i>subpictus</i>	3	
				<i>annularis</i>	81	
Dubrakudi	1940					
	March ..	Tank 9	250	<i>sundaicus</i>	1	Scanty marginal putrefying floating algæ; Lyngbya; Ceratophyllum demersum.
				<i>subpictus</i>	44	
				<i>vagus</i>	7	
	April ..	Do.	450	<i>sundaicus</i>	2	Do.
				<i>subpictus</i>	10	
	May ..	Do.	400	<i>sundaicus</i>	2	Algæ disappeared after rains in the 3rd week. Ceratophyllum demersum.
				<i>subpictus</i>	15	
	April ..	Pool near Tank 9	275	<i>sundaicus</i>	4	No weeds.
				<i>subpictus</i>	18	
..	Sept. ..	Borrowpit 14	750	<i>sundaicus</i>	1	Scanty growth of Spirogyra.
				<i>subpictus</i>	23	



TABLE XII—*contd.*

Locality.	Date.	Type of breeding place.	Salinity parts per 100,000.	Species found breeding.	Number of each species.	Type of aquatic vegetation.
Dubrakudi	1940					
	Oct. ..	Borrowpit 4 and 6A.	450	<i>sundaicus</i>	3	Scanty growth of Spirogyra, Lyngbya.
				<i>subpictus</i>	11	
				<i>annularis</i>	1	
	Nov. ..	Ricefield 12	650	<i>sundaicus</i>	10	Scanty growth of Spirogyra.
				<i>subpictus</i>	32	
	Dec. ..	Do.	850	<i>sundaicus</i>	20	Scanty growth of Lyngbya, Spirogyra.
				<i>subpictus</i>	60	
	March ..	Tank 6	300	<i>sundaicus</i>	8	Floating putrefying algæ; Lyngbya in abundance; <i>Ceratophyllum demersum</i> .
				<i>subpictus</i>	86	
	April ..	Do.	350	<i>sundaicus</i>	17	Do.
				<i>subpictus</i>	24	
	May ..	Do.	425	<i>sundaicus</i>	1	<i>Ceratophyllum demersum</i> .
				<i>subpictus</i>	20	
	Nov. ..	Do.	325	<i>sundaicus</i>	20	Putrefying marginal algæ; Lyngbya, Chætomorpha; <i>Ceratophyllum demersum</i> .
				<i>subpictus</i>	35	
	Dec. ..	Do.	250	<i>sundaicus</i>	25	Do.
				<i>subpictus</i>	40	
	1941					
	Jan. ..	Do.	250	<i>sundaicus</i>	7	Ceratophyllum, scanty growth of algæ; Lyngbya, Chætomorpha, Spirogyra.
				<i>subpictus</i>	25	
	Feb. ..	Do.	250	<i>sundaicus</i>	8	Do.
				<i>subpictus</i>	19	
	Feb. ..	Borrowpit 38	1,250	<i>sundaicus</i>	15	Putrefying algæ; Lyngbya, Oscillatoria, Anabaena.
				<i>subpictus</i>	81	
	March ..	Do.	1,250	<i>sundaicus</i>	17	Do.
				<i>subpictus</i>	79	



TABLE XII—*contd.*

Locality.	Date.	Type of breeding place.	Salinity parts per 100,000.	Species found breeding.	Number of each species.	Type of aquatic vegetation.
Dubrakudi	1941					
	March ..	Borrowpit 3A	1,200-1,400	<i>sundaicus</i>	7	Putrefying plaques of algæ, mostly Lyngbya.
				<i>subpictus</i>	79	
	March ..	Chilka Lake 1	1,050	<i>sundaicus</i>	1	Heavy growth of Potamogeton, Lyngbya, scanty growth of Cladophora.
"				<i>subpictus</i>	98	
	March ..	Chilka Canal 5	1,050	<i>sundaicus</i>	1	Halophila, heavy growth of Cladophora, scanty growth of Lyngbya.
"				<i>subpictus</i>	6	
	April ..	Do.	1,050	<i>sundaicus</i>	3	Do.
"				<i>subpictus</i>	104	
	1940					
Proyagi	Jan. ..	Chilka Lake near Fish Store.	1,300	<i>sundaicus</i>	1	Putrefying floating algæ, heavy growth of Lyngbya, Najas.
				<i>subpictus</i>	30	
"	March ..	Borrowpit between Lake and Ricefield.	400	<i>sundaicus</i>	2	Putrefying floating algæ.
				<i>subpictus</i>	28	
"	March ..	Tank between Bhamberakudi and Proyagi village.	1,150	<i>sundaicus</i>	3	Do., Scirpus, Nymphaea.
				<i>subpictus</i>	38	
"	April ..	Do.	1,275	<i>sundaicus</i>	2	Do.
				<i>subpictus</i>	23	
"	Sept. ..	Swamp near Bhamberakudi village.	75	<i>sundaicus</i>	1	<i>Ceratophyllum demersum</i> .
				<i>subpictus</i>	6	Putrefying algæ, Chætomorpha.
"	Oct. ..	Do.	200	<i>sundaicus</i>	6	Do.
				<i>subpictus</i>	9	
"	Oct. ..	Tank near Chowkidar's house, Proyagi village.	350	<i>sundaicus</i>	14	} Oscillatoria, Anabaena, Chlorella, Nostocaceæ, Chroococcus, Chlamydomonas, Spondilosium, <i>Desmidiium occidentale</i> , Ceratophyllum.
"	Nov. ..	Do.	400	<i>sundaicus</i>	23	



TABLE XII—*contd.*

Locality.	Date.	Type of breeding place.	Salinity parts per 100,000.	Species found breeding.	Number of each species.	Type of aquatic vegetation.
Proyagi	1941					
	Dec. ..	Tank near Chowkidar's house, Proyagi village.	90	<i>sundaicus</i> <i>subpictus</i>	1 3	Najas, Hydrilla.
,,	1942					
	Jan. ..	Do.	110	<i>sundaicus</i> <i>subpictus</i> <i>annularis</i>	9 6 2	Najas, Hydrilla, Ceratophyllum, Cladophora, Spirogyra.
,,	1940					
	Oct. ..	Swamp near shore of lake west of Proyagi village.	950-1,250	<i>sundaicus</i> <i>subpictus</i>	21 86	Mostly putrefying algæ, Najas.
,,	Nov. ..	Swamp near Bhamberakudi village.	1,000	<i>sundaicus</i> <i>subpictus</i>	2 58	Do.
,,	Nov. ..	Swamp near Lake, north of village.	650	<i>sundaicus</i> <i>subpictus</i>	4 81	Oscillatoria, Anabæna, Chlorella, Chlamydomonas, Ankistro desmus.
,,	Nov. ..	Swamp near Lake near fish store.	500	<i>sundaicus</i> <i>subpictus</i>	4 8	Putrefying floating algæ, Lyngbya in abundance, Najas.
,,	1941					
	Jan. ..	Swamp near Temple.	725	<i>sundaicus</i> <i>subpictus</i>	5 7	Putrefying marginal algæ, Najas.
,,	Feb. ..	Do.	1,000	<i>sundaicus</i> <i>subpictus</i>	3 9	Do.
,,	March ..	Do.	900	<i>sundaicus</i> <i>subpictus</i>	17 50	Do.
,,	Feb. ..	Swamp near village.	500	<i>sundaicus</i> <i>subpictus</i>	2 6	Putrefying marginal algæ.



TABLE XII—contd.

Locality.	Date.	Type of breeding place.	Salinity parts per 100,000.	Species found breeding.	Number of each species.	Type of aquatic vegetation.
Proyagi	1941					
	Feb. ..	Tank near ruins of Dharmasala.	300	<i>sundaicus</i>	1	Pistia, Hydrilla, Najas, scanty growth of Chaetomorpha.
				<i>annularis</i>	5	
				<i>hyrcanus</i>	4	
	Feb. ..	Pool near chowkidar's house.	50	<i>sundaicus</i>	9	Not recorded.
				<i>subpictus</i>	7	
	March ..	Pool near fish godown.	400	<i>sundaicus</i>	15	Najas, Lyngbya, Oscillatoria.
				<i>subpictus</i>	2	
"	March ..	Swamp near fish godown.	850	<i>sundaicus</i>	5	Do.
				<i>subpictus</i>	16	
Chatrapur	1941					
	Jan. ..	Tank 33	75	<i>sundaicus</i>	16	Putrefying algæ: Oscillatoria, Chlorella, Desmidium, Protococcus; Najas, Hydrilla.
				<i>subpictus</i>	16	
				<i>annularis</i>	16	
				<i>hyrcanus</i>	2	
	Feb. ..	Do.	75	<i>sundaicus</i>	19	Do.
				<i>subpictus</i>	14	
				<i>annularis</i>	9	
				<i>hyrcanus</i>	2	
	Feb. ..	Borrowpit 32	60-75	<i>sundaicus</i>	28	Putrefying algæ, scanty growth of Spirogyra, Oscillatoria, Lyngbya.
				<i>subpictus</i>	31	
				<i>vagus</i>	4	
				<i>annularis</i>	5	
"	March ..	Do.	50	<i>sundaicus</i>	1	Plaques of putrefying algæ.
				<i>subpictus</i>	2	
				<i>annularis</i>	2	



TABLE XII—*contd.*

Locality.	Date.	Type of breeding place.	Salinity parts per 100,000.	Species found breeding.	Number of each species.	Type of aquatic vegetation.
Chatrapur	1940	Tampara 1	75	<i>sundaicus</i>	1	Putrefying algæ. Najas, Hydrilla.
	Nov. ..			<i>annularis</i>	63	
				<i>vagus</i>	1	
				<i>subpictus</i>	5	
,,	1941	Do.	75	<i>sundaicus</i>	8	Najas, Hydrilla, Ceratophyllum, Chara, algæ (unidentified).
	July ..			<i>subpictus</i>	125	
				<i>annularis</i>	176	
				<i>vagus</i>	11	
,,	Aug. ..	Do.	40	<i>sundaicus</i>	4	Do.
				<i>subpictus</i>	322	
				<i>annularis</i>	205	
				<i>vagus</i>	4	
,,	1942	Tank 34	50	<i>sundaicus</i>	5	Hydrilla, Ceratophyllum, scanty Cladophora.
	March ..			<i>subpictus</i>	6	
				<i>annularis</i>	2	
Gopalpur area.	1939	Pool in Casuarina plantation near shore of creek.	90	<i>sundaicus</i>	2	Rotting leaves of Casuarina. No weeds.
	Oct. ..			<i>hyrcanus</i>	8	
,,	1940	Pool near creek on Haripur-Bandra side.	200	<i>sundaicus</i>	1	Scanty floating putrefying algæ.
	July ..			<i>subpictus</i>	10	



TABLE XII—*contd.*

Locality.	Date.	Type of breeding place.	Salinity parts per 100,000.	Species found breeding.	Number of each species.	Type of aquatic vegetation.
Gopalpur area.	1940					
	Aug. ..	Pool near creek on Haripur-Bandra side.	450	<i>sundaicus</i>	2	Scanty floating putrefying algæ.
				<i>subpictus</i>	8	
	Oct. ..	Small pit between creek and old navigation canal.	600	<i>sundaicus</i>	2	No weeds.
				<i>subpictus</i>	8	
	1941					
,,	May ..	Tank near Haripur village.	30	<i>sundaicus</i>	2	Pistia, Ceratophyllum, Spirogyra and other algæ.
				<i>subpictus</i>	1	
	July ..	Old navigation canal 6.	50	<i>sundaicus</i>	2	Hydrilla, Nymphaea, Spirogyra.
				<i>annularis</i>	2	
,,	1942					
	Jan. ..	Old navigation canal 6.	15	<i>sundaicus</i>	1	Hydrilla, Nymphaea, Spirogyra and scanty growth of Pistia.
				<i>hyrcanus</i>	32	
				<i>vagus</i>	1	
,,	1941					
	Dec. ..	Tank 39	120	<i>sundaicus</i>	12	Najas, Hydrilla, Ceratophyllum, scanty growth of Cladophora, Spirogyra.
				<i>subpictus</i>	32	
				<i>vagus</i>	3	
,,	1942					
	Jan. ..	Do.	130	<i>sundaicus</i>	34	Najas, Hydrilla, Ceratophyllum.
				<i>subpictus</i>	1	



TABLE XII—contd.

Locality.	Date.	Type of breeding place.	Salinity parts per 100,000.	Species found breeding.	Number of each species.	Type of aquatic vegetation.
Gopalpur area.	1942					
	Feb. ..	Tank 39	130	<i>sundaicus</i>	2	<i>Najas</i> , Hydrilla, Ceratophyllum.
				<i>subpictus</i>	10	
				<i>vagus</i>	5	
	Feb. ..	Pool 28	15	<i>sundaicus</i>	5	<i>Najas</i> , Hydrilla, Ceratophyllum, <i>Cladophora</i> , <i>Spirogyra</i> .
				<i>annularis</i>	19	
				<i>hyrcanus</i>	21	
				<i>aconitus</i>	3	
	March ..	Do.	15	<i>sundaicus</i>	1	Do.
				<i>annularis</i>	15	
Ganjam area				<i>hyrcanus</i>	27	
	Feb. ..	Pool 40	65	<i>sundaicus</i>	4	Hydrilla, <i>Najas</i> , <i>Cladophora</i> .
				<i>annularis</i>	3	
				<i>hyrcanus</i>	4	
	March ..	Do.	95	<i>sundaicus</i>	1	Do.
				<i>annularis</i>	24	
	1941					
	April ..	Pool 39	30	<i>sundaicus</i>	2	<i>Najas</i> .
				<i>subpictus</i>	1	
	May ..	Tank 12	100	<i>sundaicus</i>	3	Ceratophyllum, Hydrilla, <i>Najas</i> , scanty growth of <i>Spirogyra</i> , <i>Oscillatoria</i> .
				<i>subpictus</i>	1	
				<i>annularis</i>	1	
"	July ..	Do.	90	<i>sundaicus</i>	1	Hydrilla, <i>Najas</i> , Ceratophyllum, scanty growth of <i>Lyngbya</i> , <i>Spirogyra</i> .
				<i>subpictus</i>	2	
	Aug. ..	Do.	75	<i>sundaicus</i>	1	Do.
				<i>annularis</i>	4	



TABLE XII—contd.

Locality.	Date.	Type of breeding place.	Salinity parts per 100,000.	Species found breeding.	Number of each species.	Type of aquatic vegetation.
Ganjam area	1941 Nov. ..	Tahk 12	40	<i>sundaicus</i>	3	Hydrilla, Najas, Ceratophyllum, scanty growth of Lyngbya, Spirogyra.
				<i>subpictus</i>	3	
				<i>annularis</i>	31	
"	Dec. ..	Do.	40	<i>sundaicus</i>	2	Do.
				<i>annularis</i>	18	
"	1942 March ..	Do.	50	<i>sundaicus</i>	5	Hydrilla, Najas, Ceratophyllum, scanty growth of Oscillatoria.
				<i>annularis</i>	15	
				<i>subpictus</i>	10	
"	1941 Aug. ..	Pool 24	155	<i>sundaicus</i>	3	Najas, Ceratophyllum, Cladophora.
				<i>subpictus</i>	12	
"	Nov. ..	Do.	50	<i>sundaicus</i>	2	Najas, Ceratophyllum, Cladophora, Lyngbya.
				<i>subpictus</i>	15	
"	Dec. ..	Do.	95	<i>sundaicus</i>	9	Do.
				<i>subpictus</i>	14	
"	1942 Jan. ..	Do.	145	<i>sundaicus</i>	4	Do.
				<i>subpictus</i>	21	
				<i>vagus</i>	2	
				<i>annularis</i>	1	
"	Feb. ..	Do.	170	<i>sundaicus</i>	9	Do.
				<i>subpictus</i>	25	
"	March ..	Do.	275	<i>sundaicus</i>	11	Do.
				<i>subpictus</i>	11	



TABLE XII—contd.

Locality.	Date.	Type of breeding place.	Salinity parts per 100,000.	Species found breeding.	Number of each species.	Type of aquatic vegetation.
	1941					
Ganjam area	Oct. ..	Pool 8A	85	<i>sundaicus</i>	5	Marginal growth of <i>Najas</i> , <i>Cladophora</i> , <i>Lyngbya</i> .
„	Nov. ..	Do.	85	<i>sundaicus</i>	11	Do.
				<i>annularis</i>	2	
				<i>subpictus</i>	29	
„	Dec. ..	Do.	95	<i>sundaicus</i>	17	Do.
				<i>subpictus</i>	18	
	1942					
„	Jan. ..	Do.	125	<i>sundaicus</i>	24	Do.
				<i>subpictus</i>	7	
„	Feb. ..	Do.	125	<i>sundaicus</i>	15	Do.
				<i>annularis</i>	3	
				<i>subpictus</i>	11	
„	March ..	Do.	150	<i>sundaicus</i>	9	Do., but only in one corner.
				<i>subpictus</i>	15	
	1941					
„	Nov. ..	Pool 5	Not recorded.	<i>sundaicus</i>	4	Ceratophyllum, <i>Cladophora</i> , <i>Spirogyra</i> , scanty growth of <i>Hydrilla</i> .
				<i>annularis</i>	7	
„	Dec. ..	Do.	55	<i>sundaicus</i>	9	Do.
				<i>subpictus</i>	18	
	1942					
„	Jan. ..	Do.	60	<i>sundaicus</i>	18	Do.
				<i>annularis</i>	25	
				<i>pallidus</i>	2	



TABLE XII—contd.

Locality.	Date.	Type of breeding place.	Salinity parts per 100,000.	Species found breeding.	Number of each species.	Type of aquatic vegetation.
Ganjam area	1942 Feb. ..	Pool 5	70	<i>sundaicus</i>	17	Ceratophyllum, Cladophora, Spirogyra, scanty growth of Hydrilla.
				<i>annularis</i>	12	
				<i>pallidus</i>	3	
				<i>subpictus</i>	1	
,,	March ..	Do.	85	<i>sundaicus</i>	6	Do.
				<i>annularis</i>	18	
,,	1941 Aug. ..	Pool 3	45	<i>sundaicus</i>	18	Hydrilla, Ceratophyllum, putrefying algæ Lyngbya, Spirogyra.
				<i>subpictus</i>	12	
,,	Sept. ..	Do.	50	<i>sundaicus</i>	29	Do.
				<i>subpictus</i>	4	
				<i>annularis</i>	4	
,,	Oct. ..	Do.	25	<i>sundaicus</i>	16	Do.
				<i>annularis</i>	4	
				<i>subpictus</i>	1	

November and December—No breeding due to settling of algæ on account of rains.

,,	1942 Jan. ..	Pool 3	40	<i>sundaicus</i>	18	Hydrilla, Ceratophyllum, Cladophora.
				<i>annularis</i>	11	
				<i>pallidus</i>	7	
,,	Feb. ..	Do.	45	<i>sundaicus</i>	16	Do.
				<i>annularis</i>	8	
,,	March ..	Do.	85	<i>sundaicus</i>	10	Do.
				<i>annularis</i>	12	
				<i>subpictus</i>	4	



TABLE XII—concl'd.

Locality.	Date.	Type of breeding place.	Salinity parts per 100,000.	Species found breeding.	Number of each species.	Type of aquatic vegetation.
Ganjam area	1941					
	Aug. ..	Pool 6	60	<i>sundaicus</i>	39	Hydrilla, Najas, Ceratophyllum, Cladophora, Anabaena (typical <i>sundaicus</i> breeding place).
				<i>culicifacies</i>	1	
				<i>annularis</i>	17	
				<i>subpictus</i>	3	
	Sept. ..	Do.	60	<i>sundaicus</i>	36	Do.
				<i>annularis</i>	39	
				<i>subpictus</i>	14	
	Oct. ..	Do.	40	<i>sundaicus</i>	3	Do., but scanty growth due to rains.
				<i>annularis</i>	17	
	Nov. ..	Do.	40	<i>sundaicus</i>	6	Do., algae mostly at bottom of pool.
				<i>annularis</i>	28	
	Dec. ..	Do.	50	<i>sundaicus</i>	9	Do., algae re-appeared.
				<i>annularis</i>	10	
..	1942					
	Jan. ..	Do.	60	<i>sundaicus</i>	19	Do.
				<i>subpictus</i>	5	
				<i>annularis</i>	15	
	Feb. ..	Do.	70	<i>sundaicus</i>	22	Do.
				<i>annularis</i>	8	
				<i>subpictus</i>	2	
	March ..	Do.	95	<i>sundaicus</i>	10	Do.
				<i>annularis</i>	13	
				<i>subpictus</i>	9	



TABLE XIII.

*Larvæ of A. sundaicus collected from Chilka Lake south of Kalijai Island.*

Period.	Locality.	Number of adult mosquitoes hatched out.	Number of <i>A. sundaicus</i> hatched out.
<b>1940</b>			
January ..	Proyagi	31	1
November ..	Sabilia	529	1
<b>1941</b>			
January-April ..	Sabilia	964	8
March .	Dubrakudi	99	1
<b>1942</b>			
March ..	Kesopur	95	6
TOTAL ..		1,718	17

TABLE XIV.

*Dissections of anopheline mosquitoes, May 1939 to March 1942.*

Species.	NUMBER DISSECTED.					NUMBER INFECTED.	
	1939.	1940.	1941.	1942.	TOTAL.	Gut.	Gland.
<i>A. sundaicus</i> ..	150	4,836	4,285	1,443	10,714	32	51
<i>A. annularis</i> ..	6,049	6,713	7,497	585	20,844	1	0
<i>A. culicifacies</i> ..	859	1,099	317	30	2,305	0	0
<i>A. aconitus</i> ..	142	56	402	74	674	0	0
<i>A. varuna</i> ..	316	108	20	6	450	0	0



*Dissections of A. sundaicus, May 1939 to March 1942.*

[illegible]



TABLE XV—*contd.*

Month.	SANA NAIRI.			BALUGAON.			KESOTUR.			KONOKA.			SABILIA-RAMBHA.			TOLO HUMMA.			DUBRAKUDI.			
	Infected.			Infected.			Infected.			Infected.			Infected.			Infected.			Infected.			
	Dissected.	Gut.	Gland.	Dissected.	Gut.	Gland.	Dissected.	Gut.	Gland.	Dissected.	Gut.	Gland.	Dissected.	Gut.	Gland.	Dissected.	Gut.	Gland.	Dissected.	Gut.	Gland.	
1940																						
Jan.	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	
Feb.	..	..	..	..	..	..	..	..	..	2	0	0	14	0	0	..	..	..	..	4	0	0
March	3	0	0	..	..	..	..	..	..	15	0	0	211	0	0	4	0	0	4	0	0	
April	1	0	0	1	0	0	1	0	0	14	0	0	470	4	3	9	0	0	14	1	0	
May	1	0	0	3	0	0	3	0	0	1	0	0	30	0	1	7	0	0	14	0	0	
June	3	0	0	..	..	..	..	..	..	5	0	0	3	0	0	10	0	0	4	0	0	
July	1	0	0	..	..	..	..	..	..	..	..	..	..	..	..	4	0	0	1	0	0	
August	..	..	..	75	..	..	..	..	..	..	..	..	..	..	..	3	0	0	3	0	0	
Sept.	..	..	..	744	0	1	13	0	0	1	0	0	1	0	0	2	0	0	10	0	0	
Oct.	14	0	0	1,173	10	7	29	0	0	41	0	1	42	0	0	25	0	0	24	0	0	
Nov.	35	0	0	458	0	0	84	1	0	52	0	0	25	0	0	119	0	0	119	0	0	
Dec.	27	0	0	421	1	0	43	0	0	13	0	0	23	0	1	10	0	0	10	0	0	



1941												1942											
Jan.	101	0	0	..	..	..	296	1	0	44	0	0	70	1	0	18	0	0	9	0	0		
Feb.	360	1	2	..	..	..	65	5	1	4	0	0	52	0	1	4	0	0	11	0	0		
March	355	0	0	6	0	0	9	0	0	3	0	0	34	0	0	6	0	0	2	0	0		
April	237	0	0	2	0	0	..	..	..	8	0	0	1	0	0	..	..	..	1	0	0		
May	1	0	0	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..		
June	..	..	..	..	..	..	1	0	0	..	..	..	..	..	..	1	0	0	..	..	..		
July	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..		
August	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..		
Sept.	9	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..		
Oct.	30	0	0	..	..	..	11	0	0	..	..	..	..	..	..	..	..	..	..	..	..		
Nov.	428	0	0	1	0	0	40	0	1	..	..	..	6	0	0	6	0	0	..	..	..		
Dec.	347	0	0	2	0	0	94	0	0	7	0	0	5	0	0	2	0	0	..	..	..		
Jan.	232	0	1	..	..	..	22	0	0	..	..	..	..	..	..	..	..	..	..	..	..		
Feb.	137	0	1	1	0	0	13	0	1	..	..	..	..	..	..	..	..	..	..	..	..		
March	19	0	0	..	..	..	24	0	0	..	..	..	..	..	..	..	..	1	0	0	0		



**TABLE XV—*contd.***

[illegible]



[illegible]



TABLE XV—*concd.*

Month.	CHATRAPUR.			HARIPUR- BANDRA, GOPALPUR.			GANJAM AREA			PROYAGI.			KALLIKOTA.			BOLABANDH.			ICHHAPUR.			SORAN.		
	Dissected.			Dissected.			Dissected.			Dissected.			Dissected.			Dissected.			Dissected.			Dissected.		
	In- fected.	Gut.	Gland.	In- fected.	Gut.	Gland.	In- fected.	Gut.	Gland.	In- fected.	Gut.	Gland.	In- fected.	Gut.	Gland.	In- fected.	Gut.	Gland.	In- fected.	Gut.	Gland.	In- fected.	Gut.	Gland.
<b>1941</b>																								
Oct. ..	1	..	..	..	..	..	174	0	0	..	..	..	..	..	..	111	1	0	..	..	..	2	0	0
Nov. ..	..	..	..	7	0	0	213	1	4	..	..	..	..	..	..	..	..	..	136	1	1	11	0	2
Dec. ..	..	..	..	22	0	1	198	0	0	..	..	..	10	0	0	..	..	..	28	0	2	50	0	0
<b>1942</b>																								
Jan. ..	2	0	0	14	0	0	166	0	0	8	0	0	8	0	0	..	..	..	..	..	..	..	..	..
Feb. ..	2	0	0	22	0	0	192	0	1	..	..	..	6	0	0	39	0	0	..	..	..	..	..	..
March ..	..	..	..	..	..	..	68	0	1	..	..	..	6	0	0	277	0	0	24	..	..	..	..	..

*Note.*—In addition to the routine dissections shown above, 428 specimens of *A. sundicus*, collected on various occasions in villages visited for special purposes, were dissected with negative results.



TABLE XVI.

Name of village.	Number examined.	Spleen rate.	Average enlarged spleen.*	Spleen rate recorded by Fry (1912).
Garh Krishnaprasad ..	70	94	6.4	..
Khatiakudi .. ..	44	100	4.5	..
Garbai .. ..	28	96	5.9	98
Mohasa .. ..	45	87	7.8	63
Poradihi .. ..	33	88	7.5	..
Tua .. ..	22	91	7.4	79
Satpara .. ..	68	96	6.7	92
Alupatna .. ..	30	97	7.1	..
Nuagaon .. ..	45	98	6.4	100
Sipakundapatna ..	24	92	7.5	..
Arakhakud .. ..	70	11	8.4	..

\* Measurement in centimetres from apex of spleen to umbilicus.

TABLE XVII.

Distance from Bhusandpur (miles).	Name of village.	Date of visit.	Number of children examined.	Spleen rate per cent.	Collections of <i>A. sundaicus</i> adults.
0	Bhusandpur ..	25.vi.41	150	26	0
		5.xii.41	130	73	13
	Garhkharad ..	25.vi.41	10	40	0
	Kankapur ..	25.vi.41	32	50	0
	Deipur .. ..	25.vi.41	17	47	0



TABLE XVIII.

Distance from Gopalpur (miles).	Name of village.	Number of children examined.	Spleen rate per cent.	COLLECTIONS OF <i>A. sundaicus</i> .		REMARKS.
				Adults.	Larvæ.	
Towards Gaungi Tampara.						
1	Venkatrayapuram	70	1.4	0	0	
1½	Bakshipalli ..	88	26	0	0	
2½	Golabanda ..	87	7	0	0	
3½	Hattipada ..	60	8	0	0	On north bank of tampara.
5½	Kirtipur ..	34	6	0	0	On south bank of tampara.
4	Nuagolabanda ..	25	60	0	0	Between tam- para and sea coast.
Towards Berham- pore.						
1	Narayanapur ..	46	30	0	0	
4½	Dura ..	62	55	15	2*	Parasite rate 23 per cent (30 examined) all <i>P. falciparum</i> , <i>A. sundaicus</i> larvæ found in tank with sali- nity 75 parts per 100,000.
5½	Ampua ..	80	25	0	0	
6	Khodasingi ..	36	8	0	0	
7	Komapalli (Ber- hampore).	120	5	0	0	

\* Most of the larvæ collected at Dura died before hatching out.



TABLE XIX.

Group.	Village.	Distance from lake (miles).	Children examined.	Spleen rate per cent.	COLLECTIONS OF <i>A. sundaicus</i> .	
					Average per adult collection.	
					Nov.-Dec. 1941.	Jan.-March 1942.
I	Jaripada ..	0	105	52	44	..
	Rajendrapur ..	1	17	47	5	..
	Tangi .. ..	3	128	13	0	..
II	Soran .. ..	0	75	80	66	..
	Kuhuri .. ..	1½	122	41	21	..
	Digitpara ..	3	74	28	8	..
III	Sana Nairi ..	0	120	92	177	124
	Bolabandh ..	0	{	92	154	56
				110		
	Kumandol ..	0	..	..	..	..
	Iohhapur ..	1	{	33	38	34
				35		
	Chotrapur ..	1½	37	76	13	14
	Gangadharpur ..	2½	57	51	27	7
	Barapadar ..	3	..	..	3	..
	Nachuni .. ..	3	41	34	6	7
	Gorarajhori ..	3½	50	26	8	..
	Jagabandhupur ..	4½	33	27	2	..
	Salapdihi ..	5	64	14	..	1·5
	Gamai .. ..	5½	32	25	..	1
	Matapokra (Kaluniari)	5½	21	67	..	2
	Sunakhala ..	3	..	..	7	2
	Kulai .. ..	3½	..	..	..	1
	Binjhala .. ..	5	35	17	..	1
	Barapatna ..	5½	38	5	..	0
	Aetpur .. ..	7½	60	9	..	0



TABLE XIX—concl'd.

Group.	Village.	Distance from lake (miles).	Children examined.	Spleen rate per cent.	COLLECTIONS OF <i>A. sundaicus</i> .	
					Average per adult collection.	
					Nov.—Dec. 1941.	Jan.—March 1942.
IV	Lambodarpur ..	0	20	100	..	43
	Dhaunol (Dhaunlo on Ordinance map).	2	30	20	..	2
	Sunakera ..	3½	18	6	..	0
	Banpur ..	4½	90	22	..	2
	Narendrapur ..	5	..	..	..	0
	Kolathdihi ..	5½	..	..	..	0
V	Kesopur ..	0	112	98	12	6
	Kallikota ..	2	120	39	1	3
	Bhorosa ..	4	34	18	..	1
VI	Konoka ..	0	43	81	1·5	0·1
	Rambha ..	0	180	30	1	0·4
	Dhimiria ..	2	18	33	..	0
	Palodhuapalli ..	2½	36	14	..	0
	Chikili ..	6	66	89	..	10
		Distance from mouth of Rushikulya river.				
VII	Purana Bondho Pallibindo.	0	65	100	29	33
	Boropalli ..	½	15	100	13	4
	Ganjam ..	1½	124	27	0·2	2
	Karn Street (Ganjam)	2	18	11	..	..
	Kanchipur ..	2½	75	3	0·0	0



TABLE XX.

Distance south of Chilka Lake (miles).	Name of river or creek.	Name of village.	Date of visit.	Number of children examined.	Spleen rate per cent.	COLLECTIONS OF <i>A. sundaticus</i> .		Distance from coast (miles).	REMARKS.
						Adults.	Larvæ.		
33	Bahuda ..	Sonnapuram Peta.	24.x.41	100	69	0	5	0	Larvæ of <i>A. sundaticus</i> found in pools.
		Sorala ..	24.x.41	110	68	0	0	2½	
53	Mahender-Thanaya.	Va lapallam (Baruva).	11.i.42	47	64	3	0	0	
		Baruva ..	11.i.42	86	30	0	0	0	
70	Neol Rheo	Wajraf Kuttura.	10.i.42	85	12	0	0	0	
		Neol Rheo ..	10.i.42	138	7	0	0	0	
81	Bavanapada and Altada.	Bavanapada .. (Naupada).	19.i.42	75	98	1	1	0	One larva of <i>A. sundaticus</i> found in a pool. Adult SR 63 per cent (16 examined).
		Naupada ..	20.i.42	200	13	0	0	4	



TABLE XX—*concd.*

Distance south of Chilka Lake (miles).	Name of river or creek.	Name of village.	Date of visit.	Number of children examined.	Spleen rate per cent.	COLLECTIONS OF <i>A. sundavicus</i> .		Distance from coast (miles).	REMARKS.
						Adults.	Larvæ.		
103	Vamsadhara	Callingsapatam (i) Pedda .. Pallipeta.	18.i.42	32	3	0	0	0	
		(ii) Vadapal-lam.	18.i.42	105	1	0	0	0	
117	Lygulya ..	Pukillapeta and Ganagalla Peta.	17.i.42	45	7	0	0	0	
		Mofus Bandar	17.i.42	40	5	0	0	3	
140	Khandvilasa	Chintapalli (i) Fishermen's huts.	12.i.42	40	8	0	0	0	Larvæ of <i>A. culici-facies</i> found in an irrigation channel.
		(ii) Cultivators' huts.	12.i.42	11	73	0	0	1	
148	Champsavati (Nellimarla).	Konada ..	13.i.42	150	10	0	0	0	Larvæ of <i>A. stephensi</i> found in several wells.
		Konada Salt Factory.	13.i.42	14	7	0	0	0	
		Yerukonda ..	14.i.42	53	30	0	0	2	Larvæ of <i>A. culici-facies</i> found in one well.
		Nadipalli ..	15.i.42	36	33	0	0	2	



TABLE XXI.

*Effect of removal of aquatic vegetation on the breeding of A. sundaicus, Sabilia, 1941.*

Breeding place.	Salinity (parts per 100,000).	Aquatic vegetation before removal.	Dates of larval collections before removal of vegetation.	Number of <i>A. sundaicus</i> larvae hatched from collections before removal of vegetation.	Dates of larval collections after removal of vegetation.	Number of <i>A. sundaicus</i> larvae hatched from larvae collected after removal of vegetation.	REMARKS.
Tank 3	Feb. 8— 350	(i) A marginal belt of <i>Najas</i> 10 ft. broad.	Feb. 3	59	Feb. 12	Nil.	Vegetation removed Feb. 7-11.
	Feb. 22— 350		Feb. 4		Feb. 15		
	Mar. 3— 400		Feb. 7		Feb. 19		
	Mar. 19— 475	(ii) Scanty <i>Lyngbya</i> and <i>Spirogyra</i> .	Feb. 9		Feb. 21		Operations continued throughout March and April.
	Apr. 2— 500		Feb. 10		Feb. 25		
	Apr. 26— 500				Feb. 28		



TABLE XXI—*concd.*

Breeding place.	Salinity (parts per 100,000).	Aquatic vegetation before removal.	Dates of larval collections before removal of vegetation.	Number of <i>A. sundaticus</i> larvae hatched from collections before removal of vegetation.	Dates of larval collections after removal of vegetation.	Number of <i>A. sundaticus</i> hatched from larvae collected after removal of vegetation.	REMARKS.
Tank 2A	Feb. 8—500	Sheets of Chaetomorpha floating on surface.	Feb. 3	24	Feb. 17	5	Vegetation removed Feb. 13-15.
	Feb. 22—525		Feb. 4		Feb. 18	Nil	Algal masses reappeared within 12 hours.
	Mar. 3—600		Feb. 10		Feb. 19		
	Mar. 19—850		Feb. 12		Feb. 25	4	Operation repeated March 3-7 and 10-16.
	Apr. 2—1,625				March 1		
					March 3		
					March 6		
					March 12	4	Copper sulphate 1/420,000 applied on March 16 without effect.
					March 15		
					March 17	5	Copper sulphate 1/60,000 applied March 22.
					March 19		
					March 20	Nil	No further formation of algal masses.
					March 24		
					March 26		
					March 31	Nil	
					April 2		
					April 4		



<p><b>Tank 6</b></p>	<p>Feb. 22— 325  Mar. 19— 425  Apr. 2— 425  Apr. 26— 425</p>	<p>Hydrilla and  Najas. Algæ  absent.</p>	<p>Mar. 26  Mar. 28  Mar. 31  Apr. 2  Apr. 4</p>	<p>6  6</p>	<p>April 8  April 16  April 22  April 24  April 26</p>	<p>Nil</p>	<p>Vegetation removed  April 1-8.</p>
<p><b>Tank 19B</b></p>	<p>Feb. 22— 350  Mar. 19— 400  Apr. 4— 400</p>	<p>Pistia and Ceratophyllum in abundance.</p>	<p>Apr. 4  Apr. 8</p>	<p>21</p>	<p>April 16  April 18  April 22  April 24  April 26</p>	<p>Nil</p>	<p>Vegetation removed  April 8-18.</p>



*Malaria in the Coastal Belt of Orissa.*

TABLE XXII.

*Average daily adult collection of A. sundaiacus per week in  
controlled and adjacent uncontrolled areas,  
September 1941 to March 1942.*

Week ending	Comparison village (north of controlled area).	Comparison village (south of controlled area).	VILLAGES IN CONTROLLED AREA.			
	Kesopur.	Proyagi.	Konoka.	Sabilia.	Rambha.	
1941						
September 6 ..	0·0	..	0·0	1·0	0·0	
„ 13 ..	0·0	0·0	..	0·0	..	
„ 20 ..	0·0	..	..	0·0	..	
„ 27 ..	0·0	..	..	0·0	..	
October 4 ..	0·0	..	..	0·0	..	
„ 11 ..	0·0	0·0	..	0·0	0·0	
„ 18 ..	0·0	..	0·0	0·0	..	
„ 25 ..	5·0	..	..	0·0	..	
November 1 ..	6·3	..	0·0	0·0	1·0	
„ 8 ..	6·5	..	0·0	0·5	0·0	
„ 15 ..	8·7	..	0·0	0·0	0·0	
„ 22 ..	10·0	0·0	0·0	0·5	0·0	
„ 29 ..	9·5	..	..	1·0	0·0	
December 6 ..	11·7	..	0·0	0·3	0·5	
„ 13 ..	24·0	2·0	7·0	0·0	0·5	
„ 20 ..	11·0	..	2·0	0·3	2·0	
„ 27 ..	29·0	..	..	0·5	1·0	



TABLE XXII—concl'd.

Week ending			Comparison village (north of controlled area).	Comparison village (south of controlled area).	VILLAGES IN CONTROLLED AREA.		
			Kesopur.	Proyagi.	Konoka.	Sabilia.	Rambha.
1942							
January	3	..	..	..	0·0	..	0·0
„	10	..	7·5	..	0·5	0·2	0·0
„	17	..	..	..	0·0	0·2	0·0
„	24	..	5·0	..	0·0	0·0	9·0
„	31	..	8·0	11·0	0·0	0·0	0·0
February	7	..	3·5	..	0·0	0·0	0·0
„	14	..	6·0	..	0·0	0·0	1·0
„	21	..	2·0	..	0·0	0·0	0·0
„	28	..	2·0	..	0·0	0·0	1·0
March	7	..	3·0	..	0·0	0·0	0·0
„	14	..	11·0	1·0	0·0	0·0	0·0
„	21	..	8·5	..	0·0	0·0	0·0







# STUDIES ON THE BEHAVIOUR OF *ANOPHELES MINIMUS*.

## Part VII.

### FURTHER STUDIES ON THE COMPOSITION OF THE WATER IN BREEDING PLACES AND THE INFLUENCE OF ORGANIC POLLUTION.

BY

R. C. MUIRHEAD THOMSON, D.Sc.

*(Research Worker supported by the Royal Society and the London School  
of Hygiene and Tropical Medicine.)*

[August 17, 1942.]

## CONTENTS.

	PAGE.
INTRODUCTION .. .. .	595
METHODS AND GENERAL CONSIDERATIONS .. .. .	596
EXPERIMENTS WITH EARTH WELLS .. .. .	597
EXPERIMENTS WITH EGG PITS .. .. .	600
COMPOSITION OF THE WATER IN POOLS UNDER TARAPAT .. .. .	604
DISCUSSION .. .. .	607
APPENDIX .. .. .	609
SUMMARY .. .. .	609
REFERENCES .. .. .	610

## INTRODUCTION.

IN a previous communication (Thomson, 1941), a detailed study of the composition of the water in the normal breeding places of *A. minimus* showed that along the grassy edges this is characterized by its low content of organic matter as estimated by the albuminoid ammonia and acid permanganate (Tidy) figures. In the stagnant water of tanks, borrowpits and ricefields, where *A. minimus* was seldom found, the amount of organic matter was usually two or three times greater



than in clear running streams and rivers. This difference in composition, however, could not be considered as the deciding factor in the ultimate determination of the breeding place, because (a) the difference in composition between running and stagnant water was not absolute, e.g., the organic content of the water in many ricefields at the end of the rainy season was as low as that in garden drains where *A. minimus* was breeding, and (b) when *A. minimus* females were offered a choice of different natural waters in the laboratory, they failed to distinguish stream water from tank water and laid eggs quite indiscriminately.

On the other hand, experiments involving water polluted with rotting green vegetation and with the effluent of a tea factory showed that the female mosquito was sensitive to quite small amounts of this pollution in the water. Even when the quantity of this organic matter was equivalent to only 5 mg. oxygen per litre absorbed from acid permanganate, the females showed a marked avoidance in preference for clean unpolluted water. In view of the consistent behaviour observed under artificial laboratory conditions, it seemed very likely that in the field the female mosquito would be even more sensitive, not only to the organic products of deliberate pollution but also to the type of organic matter found in natural waters associated with anopheline breeding in general.

Although our previous experiments had shown that the low organic content of the water in the normal breeding places of *A. minimus* could not be the deciding factor in the choice of the breeding place, there still remained the possibility that the organic content of some surface waters might on occasions be sufficiently great in itself to render the water unattractive to the mosquito.

The present paper records the results of a field study in which an endeavour was made to obtain some idea of the point at which organic content becomes a limiting factor in the choice of breeding place, with the help of improved chemical methods. In the case of a stream breeder like *A. minimus*, it is very difficult to carry out a simple crucial field experiment, because the density of breeding is extremely irregular and seldom becomes high enough to give convincing results. Although this has been a serious obstacle in the present investigation, there is no reason why the methods and technique described should not produce more valuable results with vector species which breed in still water such as pools and borrowpits.

#### METHODS AND GENERAL CONSIDERATIONS.

In the previous paper (Thomson, 1941), it was shown that the two most useful estimates of the amount of organic matter in the water are the oxygen absorbed from acid permanganate (Tidy figure) and the albuminoid ammonia. In natural waters these two figures usually varied directly, and when both figures were available it was found convenient to combine them in an empirical formula 
$$\frac{(\text{albuminoid ammonia} \times 100) + (\text{Tidy figure} \times 10)}{2}$$
 to give an index of the 'degree of pollution'. It has already been stressed that the organic matter in water is highly complex and that all our chemical analyses only give a rough quantitative estimate, without giving much information regarding the quality of the organic



matter. For example, the organic matter present in natural waters such as tanks and ricefields is almost certainly very different from that of water polluted with rotting green vegetation or effluent from a tea factory, and it appears that the type as well as the total amount of organic matter should be taken into consideration. In the present paper, the question of quality is probably simplified by the fact that we are dealing with natural waters such as tanks, wells and streams, in which the organic matter is probably of a fairly uniform character.

In the discussion of the previous paper it was suggested that a further improvement of analysis would be to estimate the oxygen absorbed from alkaline permanganate. In alkaline permanganate, the oxidation of organic matter is more complete than in acid solution, and Butcher *et al.* (1937) considered that the ratio of the amount of oxidation with alkaline permanganate to that of acid permanganate would give a better indication of the stage of decomposition. In the present paper, therefore, the organic matter is estimated by the following analyses:—

Albuminoid ammonia.

Oxygen absorbed from acid permanganate (Tidy figure) in 4 hours at 40°C.

Oxygen absorbed from alkaline permanganate (Appendix).

For comparison with previous results, the first two analyses can be combined to give an empirical 'degree of pollution' as described above. The new alkaline permanganate titration provides us with a further figure of value, viz., the ratio  $\frac{\text{alkaline permanganate figure}}{\text{acid permanganate figure}}$ .

In the routine distillation for albuminoid ammonia we also get the figure for free and saline ammonia, which, however, we have already shown to be of little value in indicating the condition of the water, except where definite animal pollution is present. Most of the samples show the characteristics of peaty water, in which the albuminoid ammonia is in considerable excess of the free and saline ammonia.

Dissolved oxygen was estimated in certain cases, but not in waters fully exposed to sunlight where we had already shown that this figure varied considerably throughout the day and night and was, therefore, an unreliable index in itself.

Nitrites were almost invariably absent from the water sampled.

I am indebted to Prof. M. E. Delafield for much valuable information and criticism regarding methods of analysis. I am also very grateful to Dr. E. A. H. Roberts and the chemical staff at Tocklai Experimental Station for suggesting the simple alkaline permanganate titration and for assistance in preparing and checking some of the volumetric solutions. All the analyses, as before, were carried out by myself at Tocklai.

## EXPERIMENTS WITH EARTH WELLS.

*A. minimus* usually breeds in the grassy edges of running water, but in some districts in the Assam valley it breeds in shallow earth or 'kachcha' wells which



the tea garden coolies dig near their houses for drinking purposes (Plate XXXVII, fig. 1). The appearance of *A. minimus* in these wells is rather erratic, but, nevertheless, an attempt was made to find out the relation between oviposition and the composition of the water. It had already been noticed that, as a rule, the water in these wells was fresh and clean, and that *A. minimus* was seldom found in old disused wells in which there was a great deal of vegetation, and in which the water had a more peaty appearance.

For the first experiment, three wells in which *A. minimus* was frequently found breeding were selected, and at intervals during four weeks egg collections were made and samples of water were analysed. Throughout this period, the water in these wells remained clear and sparkling. The results are shown in Table I(a).

TABLE I.

Composition of the water in wells (in parts per million).

Date.	Well.	<i>A. minimus</i> eggs.	<i>A. vagus</i> eggs.	Tidy figure. Acid per- manganate.	Alkaline permanga- nate.	Ratio alkaline acid.	Free and saline ammonia.	Albuminoid ammonia.	'Degree of pollu- tion'*
23.v	..	3	x	x	0.78	3.2	4.1	..	..
30.v	..	Z	..	x	1.64	5.2	3.2	..	..
3.vi	..	Z	x	x	1.35	4.4	3.3	..	..
10.vi	..	4	x	x	0.47	2.8	5.9	0	0.285
12.vi	{	3	..	..	0.93	4.0	4.3	0	0.242
		4	x	x	1.27	4.4	3.5	..	..
19.vi	..	4	..	x	0.75	2.8	3.7	0	0.114
		34				4.0			

$$* \frac{(\text{albuminoid ammonia} \times 100) + (\text{Tidy figure} \times 10)}{2}$$

Formula refers to figures expressed in parts per 100,000.

Note.—x = eggs present.



PLATE XXXVII.



Fig. 1. Typical shallow earth 'kacheha' well in which analyses were carried out.



Fig. 2. Type of egg pits in which experiments were carried out.



PLATE XXXVIII.



Fig. 3. Tarapat (*Alpinia allughas*) as used in control by shade.



TABLE I—*concl.*

Date.	Well.	<i>A. minimus</i> eggs.	<i>A. vagus</i> eggs.	Tidy figure. Acid per- manganate.	Alkaline permanga- nate.	Ratio alkaline acid .	Free and saline ammonia.	Albuminoid ammonia.	'Degree of pollu- tion'*
(b)									
20.v	..	A. x	x	2.96	8.8	3.0	0.173	0.520	4.0
23.v	..	..	x	2.37	6.4	2.7	0	0.216	2.2
30.v	..	x	x	2.33	7.6	3.3	0	0.264	2.5
3.vi	..	x	x	2.96	8.4	2.8	0	0.363	3.3
10.vi	..	..	x	2.82	8.0	2.8	0	0.652	4.6
12.vi	..	..	..	2.97	9.2	3.1	..	..	..
19.vi	..	..	x	2.83	8.0	2.8	0	0.320	3.0
		33	266			2.9			
20.v	..	B.	x	11.74	21.6	1.8	0	2.000	15.9
23.v	..	..	x	10.30	19.2	1.9	0	1.362	11.9
30.v	..	..	x	7.41	15.6	2.1	0.222	0.666	7.0
3.vi	..	..	x	7.22	14.8	2.1	0.133	0.799	7.6
10.vi	..	..	x	8.16	16.8	2.1	0.294	1.200	10.1
12.vi	..	..	..	7.03	15.6	2.2	..	..	..
19.vi	..	..	x	6.54	14.2	2.2	..	..	..
			224			2.1			

\* (albuminoid ammonia  $\times$  100) + (Tidy figure  $\times$  10)

Formula refers to figures expressed in parts per 100,000.

Note.—x = eggs present.

In the second experiment, two wells were constructed close to each other at the side of a small open drain which ran through the coolie lines. In previous years, wells in this place had been specially favoured by *A. minimus*, eggs and larvæ being recorded on numerous occasions. In order to make both wells more attractive, the bare edges were furnished with a slight fringe of vegetation, in both cases *Polygonum*



sp., which grew along the edges of the drain. Although the two wells were equal in size and had the same type of 'grassy edge', they differed greatly in appearance, owing to the fact that they were dug in different types of soil. The water in well A remained clear and pure in appearance, while that of well B was more soupy or peaty in appearance and had obviously a greater amount of organic matter in suspension. At intervals during a period of four weeks, egg collections were made and routine analyses carried out. After that time *A. minimus* became so scarce that the experiments were discontinued. The results are shown in Table I(b).

Although the appearance of *A. minimus* in any well was never regular enough to enable strictly controlled experiments to be carried out, the following findings are suggestive:—

The water in those wells in which *A. minimus* frequently oviposited, viz., wells 3, 4, Z, and A, is characterized by low figures for acid permanganate (Tidy), albuminoid ammonia and alkaline permanganate, particularly in the case of the pure water of the first three wells. The albuminoid ammonia and the Tidy figure (and therefore the 'degree of pollution') agree well with those encountered in the normal breeding places of *A. minimus* recorded in the previous year (Thomson, 1941).

In well B, where no oviposition took place, the figures for albuminoid ammonia acid and alkaline permanganate, and 'degree of pollution' are high, although all figures gradually fall during the course of the experiment. The composition of this well resembles that of many shallow ricefields in June and July, as shown by previous figures.

The new alkaline permanganate figure furnishes additional information regarding the quality of the organic matter. In those wells in which oviposition took place, the ratio  $\frac{\text{oxygen absorbed from alkaline permanganate}}{\text{oxygen absorbed from acid permanganate}}$  is high with a mean value of 4.0 for the pure water in wells 3, 4 and Z, and of 2.9 in well A. In well B the ratio remains low with a mean value of 2.1, only varying from 1.8 to 2.2, despite considerable changes in the total amount of organic matter as estimated by the other figures.

In contrast to the behaviour of *A. minimus*, it is interesting to note that *A. vagus* was attracted to all wells, ranging from those with a very low organic content to those with a considerable amount of organic matter in the water.

As no further experiments could be carried out in these wells owing to a great falling off in the numbers of *A. minimus*, attention was turned to the grassy edged tea garden drains which were becoming the main breeding places at that time of the year.

#### EXPERIMENTS WITH EGG PITS.

In a previous communication (Thomson, 1940), it was shown that *A. minimus* would regularly oviposit in little bare-edged, shaded, pits or pools of water dug along the edges of a grassy edged stream which was the normal breeding place. In these little pools or egg pits the factors influencing oviposition in nature could be studied more satisfactorily than in the main stream, and their value in experimental work has already been demonstrated in observations on silt (Plate XXXVII, fig. 2).



A favourable garden drain was selected, and a series of shaded egg pits were made along the bank near the water edge. The idea in view was to vary the composition of the water in these pits by introducing either water or mud from tanks or ricefields, and to find out how *A. minimus* reacted to the changed conditions. Normally, the water which seeps into the egg pits is very pure with a low organic content, and two or three of such pits were used as controls. However, the appearance of *A. minimus* eggs, even in the controls, was so erratic that it was never possible to place the experiments on a strictly controlled basis. Although valuable information could be deduced from the instances where eggs were present, it was never possible to attribute the *absence* of eggs to any definite cause. However, although the experiments failed in their main objective, the results combined with those of the previous experiments on wells give us a much better idea of the range of water composition over which *A. minimus* will oviposit in nature.

The experiments were carried out in the rainy season, in July and August, and the results are shown in Table II.

TABLE II.

*Composition of water in egg pits beside garden drain. (All analyses in parts per million.)*

Date.	Egg pit.	<i>A. minimus</i> eggs present.	Tidy figure. Acid permanganate.	Alkaline permanganate.	Ratio alkaline acid.	Free and saline ammonia.	Albuminoid ammonia.	'Degree of pollution'.
15.vii	B.	..	3.73	11.2	3.0	0.266	0.750	5.6
	C.	..	3.56	10.8	3.0	0	0.570	4.6
17.vii	C.	x	3.08	8.4	2.7	0.120	0.444	3.8
	D.	x	3.50	8.4	2.4	0.750	0.480	4.2
24.vii	G.	x	2.04	7.6	3.7	0	0.600	4.0
	F.	x	3.45	10.4	3.0	..	..	..
	drain.	x	4.16	9.6	2.3	0.037	0.480	4.5
26.vii	G.	..	3.05	8.4	2.8	0.665	0.374	3.4
	C.	x	2.37	7.2	3.0	0	0.343	2.9
16.viii	F.	..	0.42	5.6	13.3	0	0.130	0.9
	G.	x	0.47	3.2	6.8	0	0.180	1.1
22.viii	G.	..	2.25	7.2	3.2	0	0.400	3.1
	drain.	..	2.38	6.0	2.5	..	..	..
27.viii	F.	..	3.63	8.8	2.4	0.347	0.775	5.6
	G.	..	2.19	6.0	2.7	0.114	0.428	3.2
29.viii	F.	..	3.39	7.2	2.1	0.332	0.773	5.5
	C.	x	2.35	6.8	2.9	0	0.600	4.1
Mean of positive egg pits	..	..	..	..	3.4			

Note.—x = eggs present.



The acid permanganate (Tidy) figures cover the range encountered in previous analyses from the garden drain itself, and show that oviposition still takes place in water with an organic content equal to the absolute maximum recorded from the stream in periods of drought (figures of 24.vii). At such a time the composition of the water with regard to organic matter is very similar to that of many tanks and ricefields where *A. minimus* does not occur.

By combining the results from wells and egg pits and tabulating the highest figures for each analysis of water in which eggs were found in nature, some idea can be obtained as to the highest organic content of the water tolerated by the ovipositing female (Table III).

TABLE III.

*Maximum figures for each separate estimate of organic matter in water in which A. minimus eggs have actually been recorded in the field.*

		Part per million.	Date.
Acid permanganate (Tidy) figure	..	4.16	24.vii
Alkaline permanganate	.. ..	10.4	24.vii
Albuminoid ammonia	.. ..	0.600	24.vii 29.viii
Free and saline ammonia	.. ..	0.750	17.vii
'Degree of pollution'	.. ..	4.5	24.vii
Ratio $\frac{\text{alkaline permanganate figure}}{\text{acid permanganate figure}}$		(lowest figure) 2.3	24.vii

Although we have no crucial experiment to go by, it seems not unlikely that the set of figures above represent conditions approaching those at which the organic content of the water becomes a limiting factor in the selection of an otherwise suitable breeding place by *A. minimus*. If we refer back to Table I(b), we see that on 30.v and 3.vi eggs were found in well A, but not in well B close by. The composition of the water in well A was well within the limits tabulated above, but that of well B on both occasions was outside the limit. As a tentative suggestion, therefore, it seems reasonable to propose that the *real* limit above which



the organic content of natural waters becomes a limiting factor in oviposition by *A. minimus* is represented by figures somewhere between those of the recorded limit tabulated in Table III and those of well B on 30.v and 3.vi. If we do this, we arrive at a set of figures which should give an approximate idea of the maximum organic content compatible with oviposition in the field (Table IV).

TABLE IV.

*Approximate composition of water above which it is suggested that organic matter becomes a limiting factor in selection of the breeding place by the female A. minimus.*

Acid permanganate (Tidy) figure* ..	6.0 parts per million.
Alkaline permanganate figure ..	12.0 parts per million.
Albuminoid ammonia .. ..	1.0 part per million.
' Degree of pollution ' .. ..	8.0
Ratio $\frac{\text{alkaline permanganate figure}}{\text{acid permanganate figure}}$	2.0 (lowest limit)

\* 4 hours at 40°C.

*Note.*—The figures for acid and alkaline permanganate limits will only be applicable when the conventions used in the estimations are the same as those adopted in this work.

In drawing up this tentative table of limiting factors, a good margin above the observed limits (Table III) has been allowed, and we, therefore, feel justified in suggesting that oviposition by *A. minimus* would seldom take place in surface waters in which the different analyses of organic matter gave higher figures than those in Table IV, or a ratio  $\frac{\text{alkaline permanganate}}{\text{acid permanganate}}$  lower than 2.0.

In the series of analyses of waters from different tanks, ricefields and borrow-pits in which *A. minimus* was never found (Thomson, 1941), it is seen that the organic content of many of these surface waters is well over the limit prescribed in Table IV, but at the same time many fall well within the limit. This seems to bear out our previous conclusions that, although *A. minimus* is more sensitive to the organic content of the water than other anophelines, such as *A. hyrcanus* and *A. vagus*, water composition is evidently not the final deciding factor in the selection of the breeding place. The fact that *A. minimus* will not oviposit in water with an organic content greater than a certain limit is sufficient in itself to explain why certain shallow ricefields, tanks, old wells, etc., are not used as breeding places, but not why *A. minimus* is also constantly absent from other tanks and ricefields in which the organic content is well within the limits tolerated.

Before going on to discuss the matter further, it is necessary to discuss a special instance in which water composition plays an important part.



## COMPOSITION OF THE WATER IN POOLS UNDER TARAPAT.

In the well-known naturalistic method of controlling *A. minimus* by shade, hedges of various plants such as *Duranta*, *Eupatorium*, *Hibiscus*, etc., are planted along each side of narrow grassy edged streams, particularly tea garden drains. Previous work (Thomson, 1940) has suggested that the breeding of this species is eliminated because the dense shade produced kills off the marginal vegetation, doing away with the zone of still water at the edges, and increasing the velocity of current to a sufficient extent to render the place unsuitable both for oviposition and for existing larvae. While this seems to be a reasonable explanation of most cases of control by shade, it is not a completely satisfactory explanation of control by shading with Tarapat. Other hedges planted along the banks convert the grassy edged stream into a bare-edged channel in a dark tunnel; but Tarapat (*Alpinia allughas*) (Plate XXXVIII. fig. 3) grows not only along the banks and edges but also in the stream itself. Although the shade of Tarapat is sufficient to eliminate the marginal vegetation, the thick stems obstruct the straight flow of water, and lead to the formation of pockets and pools of still water. The problem to be investigated was, therefore, why these shaded pools of still water alongside the stream under Tarapat were unsuitable for *A. minimus*, while shaded pools or egg pits dug in the banks of the stream were attractive and suitable breeding places.

The most obvious difference between the two types of water is that most of the pools under Tarapat contained flocculent masses of reddish ferric material, and sometimes an iridescent film on the surface, presumably due to the activities of iron bacteria. As many of these pools lay on a thick ooze of plant remains, it seemed likely that this water differed from that of egg pits mainly in the composition of the water itself, and it was hoped that the difference would be detected by means of the standard methods of analysis for organic matter. A series of analyses was, therefore, carried out (Table V).

Although the appearance of the pools under Tarapat leads us to believe that they would have a high organic content, the records show that this was not the case. On comparing Tarapat pools with egg pits there is no regular difference in any of the following tests: albuminoid ammonia, free and saline ammonia, acid permanganate, alkaline permanganate, or 'degree of pollution'. Only two of the samples from the pools had an organic content great enough to explain in itself the absence of *A. minimus*. On the other hand, the ratio  $\frac{\text{alkaline permanganate figure}}{\text{acid permanganate figure}}$  was considerably lower in the Tarapat pools than in the egg pits. In the pools the mean was 1.9, with a range of 1.6 to 2.4; while in those egg pits in which eggs were actually found the mean was 3.4, with a range of 2.3 to 6.8. This difference in the ratio almost certainly indicates a difference in the *quality* of the organic matter, a difference which might play some part in rendering the Tarapat pools unattractive.

But an even more striking difference, and one which offers a more likely explanation of the problem, is in the amount of dissolved oxygen. From the figures at the bottom of Table V it is seen that Tarapat pools, and even slowly flowing water under this type of shade, are characterized by a low oxygen content, always



TABLE V.

*Composition of water in pools under Tarapat shade.*

Date.	Tidy figure. Acid per- manganate.	Alkaline permanga- nate.	alkaline acid Ratio	Free and saline ammonia.	Albuminoid ammonia.	'Degree of pollu- tion'.
22.viii ..	3.50	8.0	2.3	0.148	0.296	3.2
	8.00	14.8	1.9	..	..	..
29.viii ..	2.65	4.8	1.8	0.125	0.347	3.1
	2.22	5.2	2.3	..	..	..
	10.91	19.5	1.8	0.952	0.656	8.7
3.ix ..	2.54	4.4	1.7	0.040	0.308	2.8
	4.49	7.2	1.6	..	..	..
	2.63	6.0	2.2	0	0.234	2.5
10.ix ..	2.70	7.2	2.6	0	0.363	3.2
	6.25	10.0	1.6	0.051	0.257	4.4
	3.71	6.4	1.7	..	..	..
17.ix ..	2.00	4.8	2.4	0	0.233	2.2
	6.04	10.0	1.7	..	..	..
	2.54	4.8	1.9	0	0.095	1.7
Mean ..	4.3	..	1.9	..	..	3.5

*Notes.*—Oxygen content of pools under Tarapat compared with that of egg pits:—

O<sup>2</sup> content of 13 pools under Tarapat varied from 0 per cent to 24 per cent saturation at the temperature of sampling. Mean was 13 per cent saturation.

O<sup>2</sup> content of 5 egg pits varied from 38 per cent to 60 per cent saturation at the temperature of sampling. Mean was 50 per cent saturation.

All samples were collected from just below the surface of the water.



considerably lower than that of the water in egg pits, and that on some occasions oxygen was absent or present only as a trace. The oxygen estimates from both types of water were unaffected by ferrous iron or by nitrites, as these were not detected in any of the samples. In a previous record of dissolved oxygen estimations (Thomson, 1941), high oxygen concentrations, frequently exceeding saturation, were encountered in different anopheline breeding places, both in rivers attractive to *A. minimus* and in tanks of still water avoided by this species; and it was concluded that dissolved oxygen played no part in the ultimate selection of the breeding place. The series of figures for pools under Tarapat, however, show an oxygen content so very much lower than any encountered previously, that it seems possible that it might constitute a limiting factor in this particular case. The next stage in the investigation of this problem would have been to find out from laboratory experiments whether or not the female *A. minimus* could distinguish the water of Tarapat pools from that of egg pits. The first difficulty encountered in this apparently straightforward experiment was that when water was removed from the pools under Tarapat it rapidly took up oxygen; and when exposed in an open dish in the laboratory, the reddish ferric material settled and the oxygen concentration soon reached 90 per cent saturation, hardly differing from egg pit water exposed in a similar dish. This rapid increase in dissolved oxygen took place even when the dish was lined with a thick layer of mud taken from the same Tarapat pool where the water was collected. No method of overcoming this difficulty was found in the short time which remained before the investigation came to an end on the departure of the author from Assam. Although the study of this special case remained incomplete, it had progressed far enough to show that there were measurable differences between the two types of water; the main characteristics of the Tarapat pools being a moderate figure for total organic matter, coupled with a low concentration of dissolved oxygen and a low ratio of alkaline permanganate titration to acid permanganate titration. Associated with these characters was the presence of flocculent masses of ferric material and iron bacteria, which are never found in egg pits or other places where *A. minimus* oviposits.

These results may also throw some light on the more general problem of iron bacteria in relation to anopheline breeding. The bacteriological side of the problem is evidently complicated, and there seems to be uncertainty regarding the number of different bacteria present and the species which are responsible for precipitating the red ferric material. The part which the bacteria themselves play in this problem would probably be extremely difficult to ascertain; and it seems more profitable, therefore, to concentrate on those characteristics of water associated with iron bacteria which can be measured, or at least estimated by fairly simple chemical means. In this investigation, we have come across several examples of important limiting factors being masked by more obvious characters, which are, however, only of secondary importance. For example, in the control of *A. minimus* by shade, the absence of light has no controlling influence by itself; and in the absence of *A. minimus* from silty streams, the silt itself is not repellent or harmful. It seems probable, therefore, that the absence of many anophelines from water with iron bacteria in evidence is not so much due to the



bacteria themselves, but to the fact that the characters and composition of the water which favour this bacterial activity are at the same time repellent to *Anopheles*.

### DISCUSSION.

In a previous communication (Thomson, 1941), it was shown that the organic content of the water is a factor of only minor importance to the larva of *A. minimus*, but one which has a very great influence on the behaviour of the female mosquito. Larvæ could be reared successfully in many different kinds of water in which they did not normally occur in nature; and in experiments with water polluted with rotting green vegetation it was shown that larvæ could successfully develop in the presence of an amount of organic pollution more than 30 times as great as that avoided by the female mosquito.

The present investigation has been concerned with extending these observations to the field, to find out how the ovipositing female reacts to the kind of organic matter present in natural waters. Although the number of positive observations is small, the experiments give us a much clearer idea of the point or region at which organic matter in natural waters probably becomes a limiting factor. Even if other conditions are favourable, an amount of organic matter in excess of this limit (Table IV) will almost certainly be sufficient in itself to make the water unattractive to the female mosquito selecting a breeding place. This is particularly well seen in shallow earth wells, which may differ considerably one from another in the quality of the water.

At this stage there might be some hesitation in accepting a statement which implies that the female mosquito is sensitive to differences in an amount of organic matter equivalent to 2 or 3 parts per million of oxygen absorbed from permanganate, or that water with a permanganate (Tidy) figure of 8 parts per million is avoided in preference to that with a figure of 4 parts per million. It is, therefore, necessary to emphasize that, in most cases, correspondingly small differences in the organic content of the water alter its appearance in a way that can be appreciated visually in the field. By looking closely at water collections, it is often possible to say at once which has the greater organic content, the colour and consistency of the water being related to the amount of organic matter, particularly as estimated by the Tidy figure. If such small differences in the amount of dissolved organic matter can be appreciated (indirectly in this case) by human sense organs, it seems reasonable to suppose that the mosquito, with its much more sensitive olfactory and chemo-receptor apparatus, is even better fitted to detect small differences in the organic content, and to use this faculty to distinguish different kinds of natural water.

*A. minimus* has specialized breeding habits, and is mainly restricted to certain kinds of water. At the conclusion of this investigation, we are not able to put forward an explanation of the selection of the breeding place which will satisfy all cases, but our experiments have shown that in many cases the absence of *A. minimus* eggs from otherwise suitable breeding places can be explained by reference to fairly simple physical factors. We have already shown in previous work that



any collection of water with bare edges fully exposed to light is unattractive, because *A. minimus* will oviposit only in shaded water collections. Similarly, the sensitivity of the *A. minimus* female to a very slight flow of water explains its absence from the edges of streams which have a straight bare edge, either naturally or rendered so by heavy shade. In addition to these, the present investigation has suggested very strongly that at certain times of the year, many ricefields, weedy tanks and borrowpits contain an amount of organic matter, as estimated by standard methods, sufficient in itself to make them unattractive to the female *A. minimus*.

Although no detailed investigation was carried out on other anophelines, figures which accumulated in the course of the experiments showed that in the field eggs of *A. hyrcanus* and *A. vagus* are deposited in water with an organic content at least twice that of the maximum tolerated by *A. minimus*.

With regard to the amount of dissolved oxygen, our previous experiments showed that there was no regular difference between river water and tank water, even though the average 'degree of pollution' was three times greater in the stagnant tank water than in the river water. In view of this and of the fact that many waters avoided by *A. minimus* still had a very high figure for dissolved oxygen, it was decided that this factor was not of primary importance. In the present investigation, we have found freshly laid eggs of *A. minimus* in an egg pit where the dissolved oxygen was as low as 47 per cent saturation at 10 a.m., and it appears, therefore, that oviposition must take place over a wide range of oxygen content. De Jesus (1941) considered that dissolved oxygen was a factor of importance in the distribution of *A. minimus* var. *flavirostris* in wells and streams, but it seems much more likely that he was really dealing with differences in the quality or quantity of organic matter.

It is possible, however, that in some cases the amount of dissolved oxygen may be so low as to constitute a limiting factor. The analyses of heavily shaded pools of water under Tarapat reveal unusual conditions, with the individual figures for permanganate titration (acid and alkaline), albuminoid ammonia and free and saline ammonia, well within the limits tolerated by *A. minimus*, but with an abnormally low figure for dissolved oxygen, this component sometimes being completely absent. In such heavily shaded pools, it appears that the oxygen used by the decomposing organic material is not so readily replaced by photosynthetic activity as in sunlit pools with abundant aquatic vegetation. The peculiar combination of characters encountered in the pools is worth further consideration, because there is some evidence to show that they may be common to heavily shaded forest pools in general. Carter and Beadle (1930), in their work on the Paraguayan Chaco, also encountered very low figures for dissolved oxygen in the tropical swamps, particularly where the shallow water was heavily shaded and protected from the action of the wind by thick clumps of vegetation. A few random analyses of jungle pools in the Nambar Forest, Assam, where *A. umbrosus* was breeding, revealed a surprisingly low 'degree of pollution' (differing little from that of *A. minimus* breeding places), considering the fact that the pools contained many dead leaves and branches.



However, the problem must rest here, until further experimental work shows whether the limiting factor in this case is really the low oxygen content, or whether it is a difference (as indicated by our analyses) in the *quality* of the organic matter which determines the behaviour of the mosquito.

## APPENDIX.

### *Alkaline permanganate method of estimating dissolved organic matter.*

This method was carried out almost exactly as described by Bottger (1938), and is much simpler in practice than it appears in print. Once the necessary solutions have been assembled, many samples of water can be analysed in a comparatively short time. With the fairly pure waters sampled in the present investigation M/100 permanganate was added to the sample, and back titrated with M/500 permanganate. With more heavily polluted waters permanganate solutions ten times stronger would be more suitable. The following example will show how the results are calculated:—

Twenty c.c. of well water was used for the analysis, and to this 20 c.c. of M/100 permanganate was added. After the usual treatment this was back titrated with M/500 permanganate in acid solution.

Blank titration consumed	..	12.9 c.c. M/500 permanganate
Well water consumed	..	14.7 c.c. M/500 permanganate
∴ Organic matter in well water used		1.8 c.c. M/500 permanganate
	i.e.,	1.8 c.c. M/100 alkaline permanganate

1 litre of M permanganate is equivalent to 8,000 mg. of oxygen

∴ 1 c.c. M/100 permanganate is equivalent to 0.08 mg. of oxygen

∴ 1.8 c.c. permanganate is equivalent to  $1.8 \times 0.08$  mg. of oxygen.

As sample was 20 c.c.,

$$\text{mg. of oxygen per litre is } 1.8 \times 0.08 \times \frac{1000}{20} = 7.2$$

∴ Oxygen absorbed from alkaline permanganate is 7.2 mg. per litre, or 7.2 parts per million.

(In this case the acid permanganate figure—4 hours at 40°C.—of the same water was 1.45 parts per million, showing that the oxygen absorbed in alkaline solution was about 5 times greater than in acid solution.)

## SUMMARY.

1. Previous studies on the dissolved organic matter of the water, and its influence on the behaviour of *A. minimus* have been extended to a study of oviposition in the field.

2. By means of an additional analytical method (the oxygen absorbed from *alkaline* permanganate), some information about the quality of the organic matter has been obtained.



3. Analysis of water in earth wells and egg pits has given further information as to the composition of water in which eggs are actually laid in nature.

4. It is suggested tentatively that the point at which organic content of the water becomes a limiting factor in oviposition, is represented approximately by the following composition :—

Oxygen absorbed from acid permanganate (Tidy figure) in 4 hours at 40°C.	..	6.0 parts per million
Oxygen absorbed from alkaline permanganate	12.0 parts per million	
Albuminoid ammonia .. .. .	1.0 part per million	
'Degree of pollution' .. .. .	8.0	
Ratio $\frac{\text{alkaline permanganate figure}}{\text{acid permanganate figure}}$ .. .. .	2.0	

An organic content which gives a higher figure than the first four of these estimates, or a ratio  $\frac{\text{alkaline permanganate figure}}{\text{acid permanganate figure}}$  less than 2.0, is probably sufficient in itself to repel the female mosquito from an otherwise suitable breeding place.

5. Field experiments confirm and extend previous conclusions about the influence of organic matter on the selection of the breeding place. The organic content of the water in many stagnant ricefields, and in some tanks and borrowpits at certain times of the year, is sufficiently high in itself to explain the continuous absence of *A. minimus*. But many other ricefields and collections of stagnant water similarly avoided by *A. minimus*, have an organic content well within the range tolerated by the female mosquito.

6. The water in heavily shaded pools under Tarapat (a 'shade' plant) shows the following characteristics with regard to dissolved organic matter :—A low or moderate figure for acid and alkaline permanganate titrations, and for albuminoid ammonia, coupled with a low ratio  $\frac{\text{alkaline permanganate figure}}{\text{acid permanganate figure}}$ , and an unusually low figure for dissolved oxygen. In some pools, dissolved oxygen is practically absent and as such may possibly be a limiting factor in this case.

## REFERENCES.

- BOTTGER, W. (1938) .. .. . Newer methods of volumetric chemical analysis. Chapman and Hall, London.
- BUTCHER, R. W., LONGWELL, J., and PENTLELOW, F. T. K. (1937) .. .. . Survey of the River Tees, Part III. Dep. Sci. Ind. Res. Water pollution research.
- CARTER, G. S., and BEADLE, L. C. (1930) .. .. . *J. Linn. Soc. Zool.*, **37**, pp. 206-258.
- DE JESUS, P. I. (1941) .. .. . *Acta. Med. Philippina*, **2**, pp. 333-340.
- THOMSON, R. C. M. (1940) .. .. . *J. Mal. Inst. Ind.*, **3**, 2 & 3, pp. 265-348.
- Idem* (1941) .. .. . *Ibid.*, **4**, 1, pp. 63-102.



## STUDIES ON THE BEHAVIOUR OF *ANOPHELES MINIMUS*.

### Part VIII.

#### THE NATURALISTIC CONTROL OF *A. MINIMUS* IN SHALLOW EARTH WELLS.

BY

R. C. MUIRHEAD THOMSON, D.Sc.

(Research Worker supported by the Royal Society and the London School of Hygiene and Tropical Medicine.)

[August 17, 1942.]

IN most parts of Assam and North Bengal continuous breeding of *A. minimus* is associated with the grassy edges of slow-flowing streams and rivers. But, in some districts, semi-permanent collections of clear unpolluted stagnant water may be used fairly regularly as breeding places. In the Dooars of North Bengal, *A. minimus* larvæ are frequently found in the clear water of grassy-edged borrowpits during cold wet spells (Ramsay and Macdonald, 1936; Harrison and Ramsay, 1933). Such breeding places are controlled by pollution with cut jungle or green vegetation (Ramsay, personal communication), and the probable way in which this method operates has been described by the present author (Thomson, 1941).

In the Assam valley a problem is created in some districts by *A. minimus* breeding in 'kachcha' wells, i.e., shallow earth wells, which tea garden coolies dig near their houses for drinking purposes. These wells are usually circular, about 5 or 6 feet in diameter, with the water level about 1 to 4 feet below the surface of the ground (Part VII, Plate XXXVII, fig. 1). The earth sides may be steep or sloping, bare-edged or fringed with varying amounts of vegetation. In these wells which are being used for drinking purposes, the water is clear and unpolluted, but older disused wells have usually more vegetation round the edges, and contain water with a certain amount of organic pollution.

The chemical properties of the water in the different wells have been dealt with in Part VII of this series of papers (Thomson, 1942). These results helped to explain why *A. minimus* was absent from the more heavily polluted wells, but



at the same time there seemed to be little prospect of making practical use of these findings for the control of wells which are actually being used. In fact, any interference with the water itself would be strongly opposed by the coolies and could not be considered practicable, and we must, therefore, look elsewhere for a more feasible method of control.

Most of the wells are dug in low-lying ground, near the edge of a stream or open drain, so as to ensure a supply of water throughout the year. Breeding in such wells is liable to be continuous and dense, particularly in the early months of the year (February to June) and in the latter part of the year after the monsoon. During the rainy season in July and August, the numbers of larvæ found there fall off considerably, to rise again to a maximum in October and November.

At present, there is no satisfactory method of controlling mosquito breeding in these wells, and even the drastic measure of filling them only leads to the appearance of new wells elsewhere.

However, such wells can be fairly simply controlled by a naturalistic method based on empirical observations in conjunction with previous findings on the behaviour of *A. minimus*. In the first two papers of this series (Thomson, 1940), it was shown that *A. minimus* prefers to oviposit in a shady situation, and that once the female has selected a breeding place it is attracted to a dark shaded site such as is formed by a thick grassy edge or a dark pocket or pit in the bank of the stream. It was also shown that *A. minimus*, although it is attracted to streams or running water, actually lays its eggs in still water at the edges. If we clear the vegetation from the edges of a stream and expose the bare edges to sunlight, *A. minimus* is prevented from ovipositing, owing to the absence of shade and the increased water movement at the margins.

If we apply these findings to the breeding of *A. minimus* in wells, it appears that the removal of the grassy edge will have no effect on water movement, and that to be effective, therefore, this will have to be accompanied by exposure of the bare edges to light; and this is precisely what can be done in wells to ensure control.

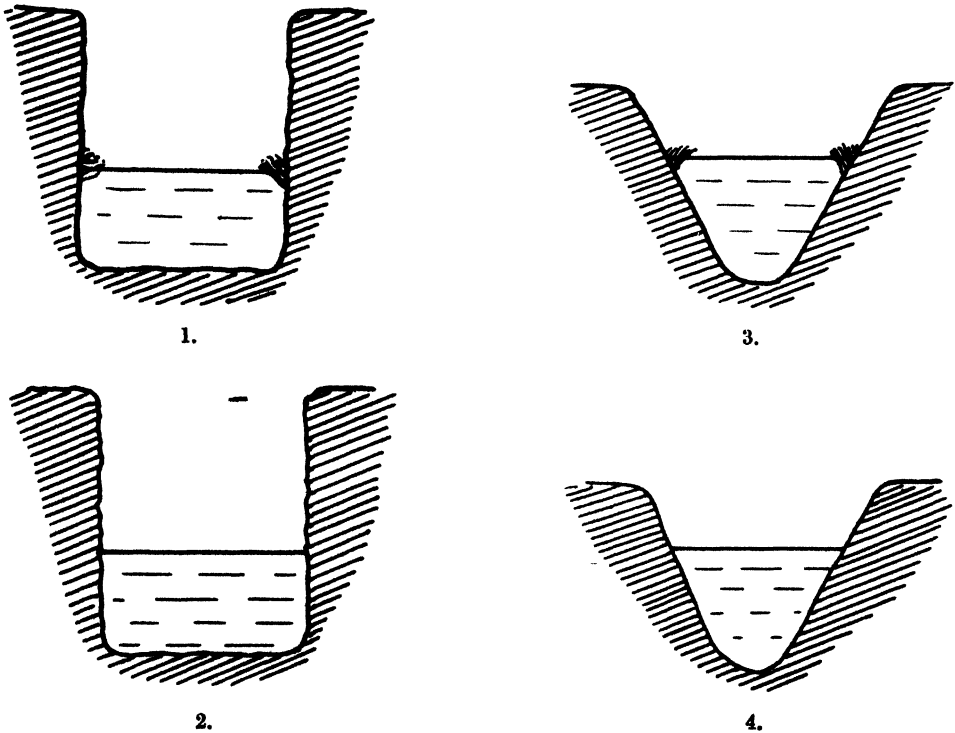
Figures 1 to 4 represent diagrams of four different types of well found in this part of Assam. In Figure 1 the earth sides are vertical and there is a fringe of vegetation round the water edge. This type is very suitable for *A. minimus*, and several hundred larvæ may be found in such a well. If we remove the grassy edge to produce the condition seen in Figure 2, breeding will still continue, although to a less extent, because the water edges are sufficiently shaded by the vertical or overhanging banks to attract the female *A. minimus*.

In Figure 3 is shown a well with sloping banks, where *A. minimus* is found breeding provided a good grassy edge exists. If this grassy edge is removed and the bare edge left fully exposed to light (Fig. 4), the well immediately becomes unsuitable for egg laying, particularly if the water surface is very near ground level. In the deeper wells a few eggs may occasionally be found, but this does not detract from the general efficiency of the control method.



The same well may pass through the different stages described above. For example a newly dug well is like Figure 4 with bare sloping edges. Although the water is very clear and unpolluted, only eggs of *A. kochi* and *A. vagus* are normally found. Later, vegetation appears at the edges and eggs of *A. minimus* may be collected regularly. In the dry weather the well may be deepened by a few feet to reach the water table, producing the type shown in Figure 2. At this stage eggs of *A. minimus* are still found, and the well becomes even more attractive when a fringe of vegetation appears as in Figure 1.

Figures.

Different types of earth wells in relation to breeding of *A. minimus*.

There is no reason why all earth wells should not conform to the type shown in Figure 4, with sloping earth walls and bare edges fully exposed to light. If the walls are beaten flat and smooth so that no little shaded pockets or holes can exist at the water edge, control of *A. minimus* should be almost perfect.

When the edges of a stream are clean-weeded and exposed to light, the modification not only prevents oviposition from taking place, but it also makes it difficult for existing larvæ to maintain their position in face of increased water



movement. In the well, however, the control effect is entirely due to the fact that the breeding place is now unsuitable for egg laying. The modification has little effect on any larvæ of *A. minimus* which are already present in the water, and larvæ may, therefore, still be found in the well for a few days after the vegetation has been removed and the edges exposed to light. When this generation of larvæ has died or reached maturity, the full effect of this control measure will become apparent.

Other anophelines likely to occur in this kind of breeding place, such as *A. vagus*, *A. kochi*, and probably *A. culicifacies*, would be unaffected by this treatment, and the method of control described above is directed towards *A. minimus* alone, being based entirely on the particular likes and dislikes of that mosquito. At the same time, it would be well worth repeating these experiments with the closely related *A. varuna*, which makes use of stagnant water collections to a much greater extent than *A. minimus*, and which may possibly have somewhat similar reactions to light and shade.

#### SUMMARY.

A simple method of controlling the breeding of *A. minimus* in shallow 'kachcha' wells is described. It depends on the fact that *A. minimus* will not oviposit along a bare edge which is fully exposed to light. By removing all vegetation from the water edge, and converting vertical earth walls into smooth sloping ones, almost perfect control is brought about. As there is no interference with the water itself, the method is particularly useful in wells used for drinking purposes.

#### REFERENCES.

- |   |   |
|---|---|
| HARRISON, C. C., and RAMSAY, G. C. (1933) | <i>J. Trop. Med. Hyg.</i> , <b>36</b> , 3, pp. 33-41. |
| RAMSAY, G. C., and MACDONALD, G. (1936)   | <i>Ind. Med. Gaz.</i> , <b>71</b> , pp. 699-710.      |
| THOMSON, R. C. M. (1940) .. ..            | <i>J. Mal. Inst. Ind.</i> , <b>3</b> , pp. 265-348.   |
| <i>Idem</i> (1941) .. ..                  | <i>Ibid.</i> , <b>4</b> , 1, pp. 63-102.              |
| <i>Idem</i> (1942) .. ..                  | <i>Ibid.</i> , <b>4</b> , 4, pp. 595-610.             |



## A DEMONSTRATION PROJECT IN THE CONTROL OF RURAL IRRIGATION MALARIA BY ANTILARVAL MEASURES.\*

BY

FRED W. KNIPE

AND

PAUL F. RUSSELL.

[September 10, 1942.]

### INTRODUCTION.

THIS paper reports on methods, costs, and results of a successful four-year experiment in the control of irrigation malaria in South India by antilarval measures. It serves as a useful contrast to experiments in malaria control by spray-killing of adult mosquitoes, reported by the authors from similar villages in the same taluk (Russell and Knipe, 1939; 1940; 1941).

The taluk, in which the project was located, is one that has become malarious since 1933-34 when a new irrigation project was opened. Epidemiology of malaria, preferential breeding habits of *A. culicifacies*, the vector, and general aspects of the relationship between malaria and irrigation in this area have all been fully discussed (Russell *et al.*, 1938; Russell and T. R. Rao, 1940a, 1940b, 1941; Russell and H. R. Rao, 1940; Russell, 1938).

### DESCRIPTION OF AREA.

The project was confined to the area known as Senjayakollai, actually a collection of well-defined rural village hamlets, one of which bears the area name. (In the

---

\* This project was carried out under the auspices, and with the support, of the International Health Division of the Rockefeller Foundation co-operating with the Madras Health Department and the Pasteur Institute of Southern India. The authors are grateful for the helpful assistance of Mr. T. Ramachandra Rao, Dr. M. Kumara Menon and Mr. N. R. Sitapathy.



## 616 *Control of Rural Irrigation Malaria by Antilarval Measures.*

text of this article the name, unless an exception is noted, always refers to the whole area. In the tables, only the hamlet is included under the name). The six villages in the Senjayakollai area, and their 1931 populations, were as follows :—

Enadi ..	.. 1,440	Naravallikkollai ..	.. 400
Aladikumalai ..	.. 550	Surankadu ..	.. 300
Senjayakollai ..	.. 500	Veerakurichchi ..	.. 200

The total population was, therefore, approximately 3,390, and, since the area was about 7 square miles, the density was about 480 persons per square mile.

The area was criss-crossed by numerous main, sub-main and secondary or branch canals of the newly developed irrigation system mentioned above. There were 3·3 miles of main canal with one drop.\* The main canal fed 4·6 miles of branch canals, and 8 drops occurred on these canals. In turn, branch canals fed many miles of poorly designed and maintained field channels.

Within the area were a total of 27 tanks, varying in size from a few hundred square feet to several thousand square feet. These were reservoirs with earth bunds, built before the new canal system was introduced. Some were shallow over the greater part of their area, thus allowing enormous 'marginal edges' for mosquito breeding. Others were sufficiently deep to keep down surface vegetation over most of the wetted area.

One small tank formed the centre of a swamp. The surrounding low land was under water of varying depth during the year. Most of this land was planted with rice when water was not too deep, as it usually was if a heavy rain fell immediately before planting time. The wetted area was about 175 acres in extent. Surface run-off from it was through a narrow outlet which frequently became clogged with sand. After clogging there would be no run-off until water backed up sufficiently high to overflow and wash away the obstruction. Gradient was practically flat throughout the length of the drain—about 3 miles. Therefore, accumulated rainfall and waste irrigation water flowed off very slowly.

West of this swamp was the only real seepage within the area, and it probably resulted from a combination of very porous soil and an abundance of irrigation water coming from a higher level. Since this seepage was not extensive and never appeared to be a dangerous source of mosquitoes, it was not eliminated.

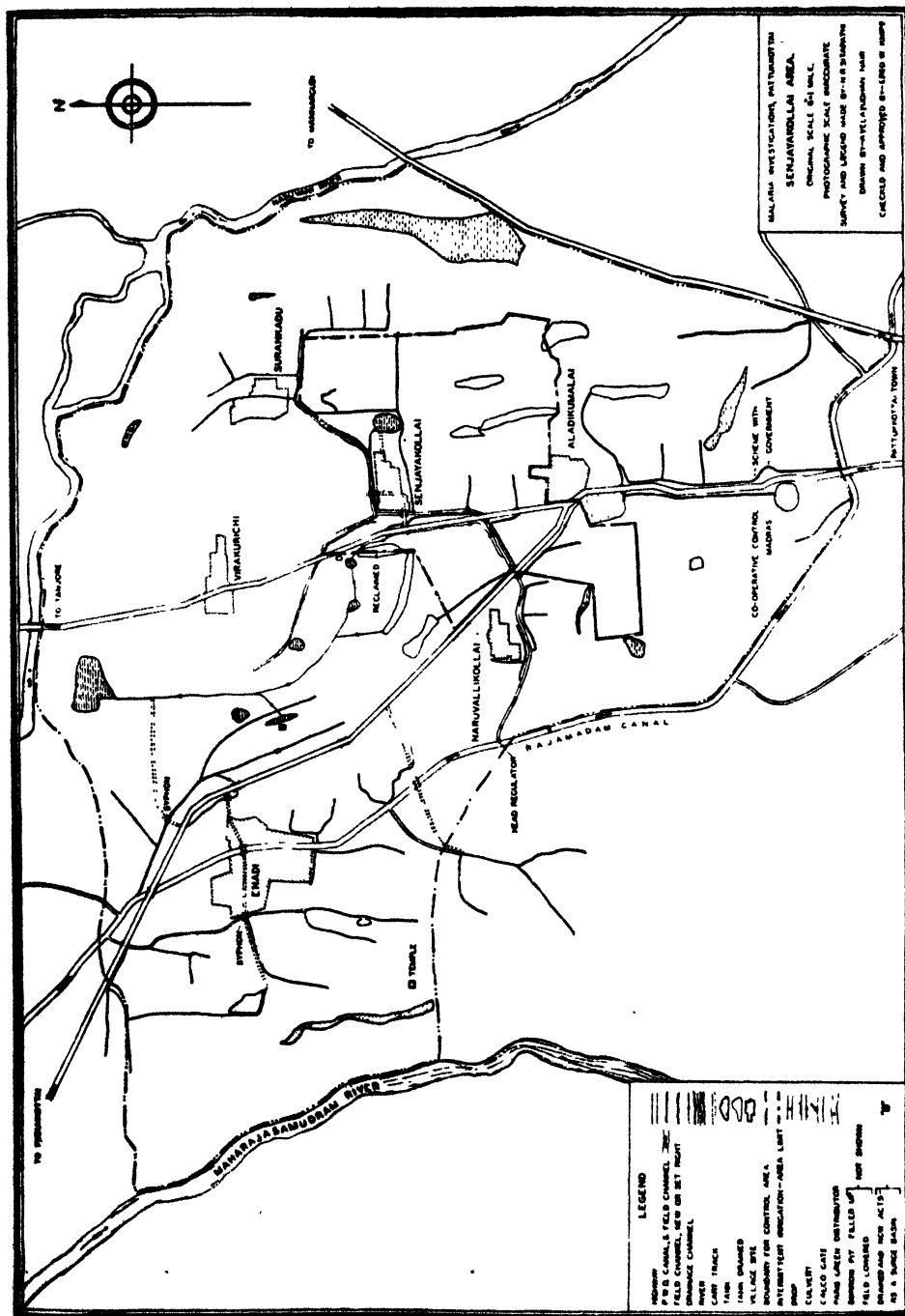
Because this was an irrigated area, it might be supposed that no irrigation wells would be required. Quite the contrary was true. Canal irrigation water was used for rice between June and January. Between January and June, a crop of ground-nuts (pea-nuts) was grown and subsoil water from wells used to irrigate it. There were about 1,300 wells within the area, used almost exclusively for irrigation. There were 110 domestic but non-drinking-water wells, and 40 drinking-water wells, for the supply of the six villages.

---

\* A 'drop' in a canal is a term applied to a structure over which water falls from one elevation to another. Its purpose is to reduce gradient on a given length of canal. [It is also known as a 'fall'—*Ed.*]



**MAP.**



**Senjayakollai project area.**



## 618 *Control of Rural Irrigation Malaria by Antilarval Measures.*

The 7 square miles (4,480 acres) could be classified approximately as follows:—

1	Village sites .. .. .	130 acres.
2	Tanks and tank beds .. .. .	250 „
3	Swamp .. 175 acres .. .. .	*
4	Seepage .. 25 „ .. .. .	*
5	Wooded land .. .. .	60 „
6	Irrigated land .. .. .	1,900 „
7	Dry crop land (too high to be irrigated) .. .. .	800 „
8	Waste land (most of this could be cultivated) .. .. .	1,190 „
9	Canals (including right of way) .. .. .	95 „
10	Highways (including right of way) .. .. .	55 „
	TOTAL .. .. .	4,480 „

\* Acreage included under irrigated land, item 6.

It should be noted that 42 per cent of the total area was irrigated, a percentage that will be reached for the entire Cauvery-Mettur project when it is fully developed.

The area seemed typical of the newly irrigated Pattukkottai taluk, with waste irrigation water in all low-lying places during the season, with countless borrowpit nurseries for *A. culicifacies*, the vector, with the usual long-standing wet fallow fields, with many shallow wells and with all the aspects of 'untidy irrigation', described elsewhere (Russell, 1938). Certainly, this area was not better, but probably somewhat worse, than average as regards malariogenic water.

It was decided to attempt to control malaria in this area by antilarval measures, and these included: filling and draining, tidying of channels, use of fishes and organic pollution, use of paris green by dustless and by automatic methods, intermittent irrigation. The work will be described by type of breeding place.



## BORROWPITS.

There are four principal methods of eliminating mosquito breeding in borrow-pits: (a) filling, (b) draining, (c) using naturalistic measures, and (d) using larvicides, such as paris green or oil. The first of these methods is to be preferred where feasible. Filling a borrowpit eliminates it as a breeding place. Draining a pit may be effective, but maintenance of the drainage channel is always necessary. One might make it a rule never to drain a borrowpit if it can be filled economically. The third method, naturalistic control, is at best still a make-shift. It may be desirable to employ such a method in the early stages of a project. But where more permanent control is desired at a minimum of recurring maintenance cost, it is better not to depend upon naturalistic methods for borrowpits. The same statement applies to the application of paris green or oil.

Within the Senjayakollai area there were hundreds of pits, some small, others very large. Since vector breeding in them was intense and they served no useful purpose and were sometimes a menace to the maintenance of canal bunds and highways, the proper way to deal with them seemed to be to eliminate them. This was not always easy because of the facts which sometimes made their original creation necessary.

Drainage of pits, undesirable at best, was, in this area, practically impossible. Suitable naturalistic control methods had not been developed. It was hoped that the application of paris green over such an extensive area could be avoided. The remaining method was filling. But some pits were very large, so that the problem of finding sufficient material for fill arose. It was solved by using fill obtained from canal maintenance, and more especially by that obtained from lands slightly above irrigation water level.

Obviously, in filling borrowpits adjacent to an irrigation canal, it is good practice to put the irrigation canal (main, branch, or field channel) in order at the same time. Some fill was obtained when canal bunds were trimmed or field channels realigned. But a far more productive source of fill from main and branch canals was the debris (sand and silt) which had been deposited in canal beds. These deposits are always a problem on every irrigation project anywhere in the world. Therefore, it seemed good practice to remove such accumulations for borrowpit fills.

Borrowpits may be dug in canal beds where additional fill is required, provided discretion is practised. These pits do not become a malaria nuisance during the irrigation season because they are within the confines of the canal bed, and, if intelligently excavated, they become filled with more sand and silt within a reasonably short time. By obtaining fills from the spoil of canal maintenance we accomplished three objectives at one operation: (a) canal beds were freed from accumulated obstruction, (b) canal bunds were maintained, and (c) mosquito-breeding borrowpits were eliminated.

However, our most productive source of fill was land slightly above irrigation water level. By using this we accomplished two objectives: (a) pit filling, and (b) lowering of fields to the point where they could be irrigated.



Certain fields, lying near a branch canal or field channel, were a few inches too high to be irrigated. Such fields, being naturally deprived of water, always tend to make their owners discontented with an irrigation system. The owner can no longer grow dry crops because the land is too wet, due to capillary rise of water through the soil, and he cannot grow wet crops because he cannot get sufficient water up to his land by canal flow. Often he cannot afford to lower his field because of labour costs. There were numerous ryots in the Senjayakollai area with this cause for discontent.

Since earth was needed to fill pits and since nearby fields were only three inches to one foot above irrigation water level, it seemed obvious that 'borrow' for filling pits could be taken from these high fields. This practice was followed throughout the area, with the result that 12·5 acres of land were lowered and placed under irrigation. Incidentally, it might be added that about five ryots per acre of land reclaimed were thus made more contented with the irrigation system.

During the period of approximately three years from May 1938 until October 1940, more than 55,000 cubic yards of earth was excavated and transported by head load for filling borrowpits (Table III). All of this earth came from the sources enumerated. All of it—by being removed from one location to another—accomplished at least one useful end, in addition to the control of mosquito breeding. Either irrigation canals benefited through maintenance, or fields were lowered to the point where irrigation was possible.

### FIELD CHANNELS.

During the process of borrowpit filling, many field channels were created where a connecting string of pits had served as a channel before. Other field channels were realigned, or, in some cases, unnecessary field channels were eliminated altogether. At the conclusion of the project, it was estimated that there was slightly less length of field channel in the area than at the beginning. There was undoubtedly less available marginal edge for mosquito breeding than in the beginning.

Field channels, under irrigation practice in the area, were by tradition the responsibility not of Government but of the ryot, both for construction and for maintenance. Hence they were in a chaotic condition, as would be expected. An effort was made to better the situation.

One vexing problem, here as elsewhere, was the control of water where a field channel divided. Never had there been any form of diversion gate or chamber at these points. Consequently, such division points usually resembled small lakes, because of the flooded areas around them. Diversion of water was usually accomplished by the ryot damming one channel with earth, while water flowed into the other. This created a maintenance problem and was also a constant source of dispute between ryots.



PLATE XXXIX.



Fig. 1. Canal-side borrowpit in Senjayakollai hamlet before filling.



Fig. 2. Same canal-side site shown in Fig. 1, as it appeared after filling the borrowpit.



PLATE XL.



Fig. 3. Culvert over canal in Senjayakollai hamlet provided so that ryots could take their carts to main highway without breaching canal banks.



Fig. 4. Site in Senjayakollai hamlet where fields at left of ditch have been lowered and the earth used to fill what was a canal borrowpit on the right of the ditch. Fields now yield a good crop of rice which were formerly too high to irrigate, but too low for good dry crops.



In order to overcome this diversion problem, as well as to assist intermittent irrigation at a later time, locally made reinforced concrete gate frames were installed at all important branch channel division points. These frames resembled a giant letter 'H' in construction, with the cross member of the 'H' slightly below the centre of the letter. Then an additional cross member was added to the top horns of the 'H'. A slot was left in this top member, and grooves were cast into the side horns. A concrete 'gate' was introduced through the slot into the grooves, thus making a very substantial and suitable diversion gate for use on side field channels. The lower horns were embedded in earth up to the cross-bar of the 'H', this cross-bar being set flush with the grade lines. In case it was necessary to block the flow of water in any channel, two of these 'H' gates were placed in position, one at any convenient angle to the other. Thus with two gates in place, flow of water in either branch of field channel could be quite easily and uniformly controlled. All water could be confined to the field channel, making maintenance much easier.

When these gates were first installed, a typical ryot reaction occurred. No ryot ever accepts anything new willingly and objections to gates were numerous. But within a few weeks all ryots in the area were demanding such gates because they had quickly recognized the advantages. Subsequently, such drainage gates were installed at all important field channel diversions. The ryots manipulated the gates themselves, and without dispute.

Field channels are recognized everywhere as potential mosquito-breeding places. In order to control breeding in them, the application of paris green by a dustless method was introduced in 1938, and was continued until intermittent irrigation was put into effect in 1940. Paris green, applied once weekly, effectively inhibited breeding in all field channels (Barber *et al.*, 1936; Russell *et al.*, 1940).

At this point it may be noted that one small, but fairly long, open drainage channel, that had been dug to drain away seepage water, also had to be treated with paris green throughout the irrigation season. This was the only place where paris green was used after intermittent irrigation was started, excepting in main or branch irrigation canals. But now that the malaria rate has been brought to a low figure, it is doubtful whether this drainage canal will produce enough mosquitoes to be dangerous, provided intermittent irrigation is effectively carried out.

## WELLS.

Wells in this area were important breeding places of *A. culicifacies*. In the days before canal irrigation was introduced, breeding in wells could have been of no consequence, for the water surface must have averaged about 45 feet below ground. *A. culicifacies*, we have observed, will not breed at that depth. But, since the coming of irrigation, the subsoil water table has risen until it now is approximately at the ground surface during the wettest season. The breeding of



*A. culicifacies* in such wells is now of considerable importance, especially during the irrigation season, when wells are not being used.

Three different methods were tried for controlling mosquito breeding in wells. They were: (a) use of paris green, (b) use of *Gambusia* fish, and (c) the naturalistic method of polluting with rotting cactus.

Paris green was first used because of its known value, but it was relatively expensive and weekly applications were necessary, and therefore some other method of control was sought.

Larvicidal fish (*Gambusia affinis*) were then tried and these completely stopped breeding where they were introduced in sufficient numbers, usually at a rate of about 25 fish per well. In order to establish *Gambusia* in wells, it was necessary to destroy all other fishes in them by treating each well with tropical chloride of lime. Some days after the other fishes had been killed by the action of the lime, *Gambusia* would be introduced. Invariably they thrived, increasing in numbers, and keeping development of mosquito larvæ to a minimum.

But, with the coming of another irrigation season, fresh irrigation water invariably found its way into the wells, and with that water always came some predatory fishes. The *Gambusia* usually disappeared soon afterwards. Wherever it was possible to protect a well from fresh irrigation water, the *Gambusia* continued to thrive. Some wells have been stocked only once in three years. Such wells were used by the fish patrol as sources of *Gambusia* stock.

We were convinced that *Gambusia* would thrive in the area and that they effectively destroyed mosquito larvæ in wells. But natural enemies preyed on them so that they were unable to establish themselves permanently in most wells or in tanks. Possibly they may in time become established in this area, as is the case in parts of Mysore State.

Anopheline mosquitoes do not usually breed in organically polluted water, so the naturalistic method of pollution of agricultural wells was tried (Hackett *et al.*, 1938; Russell, 1941). Selected wells were treated with chopped cactus, and trials indicated that the species *Opuntia dillenii* so thoroughly polluted the water that no *A. culicifacies* would breed in it for periods of from six to eight weeks. This cactus was abundant in the area. Selected wells were stocked with it once every four or five weeks. The great disadvantage in the use of this organic pollution was that enormous numbers of culicine mosquitoes developed in the wells during the initial stages of the cactus decay. After about ten days, however, even culicines were kept away. Good control lasted generally for about three weeks.

### TANKS.

There were 27 tanks within the area. Probably only three or four of these really served a useful purpose, but all of them bred *A. culicifacies* to some extent. It is probable that the output of this vector species from tanks was, by itself, not



dangerous. There was no malaria before the new canal irrigation was introduced, although the tanks had long been in existence. But it seemed advisable to reduce the mosquito population coming from them as much as possible. Five tanks were eliminated, either by drainage or by filling, and much of the land reclaimed was turned into productive ricefields (about 10 acres). The largest tank bed to be drained (near Enadi village) now acts as a surge basin during the monsoon and is not cultivated. Formerly, surface run-off filled this tank, broke the bunds and flooded a considerable area each year. This happened so frequently that rice culture below the bund had been largely abandoned. Now there is a permanent drainage system, which keeps the tank bed dry during normal times. When heavy rains occur, the tank bed acts as a surge basin which again dries slowly. The bunds do not breach and fields below are not flooded. This method of taking care of excess water has returned several acres to cultivation and has eliminated mosquito breeding as well.

The tank which served the hamlet of Senjayakollai required special treatment. It could not be eliminated. During the hot season of 1940, this tank bed dried completely. It was then thoroughly cleaned and good marginal edges were established. Later, water was introduced into it from the irrigation canal, special care being taken to avoid the introduction of native fish. *Gambusia* were then introduced into the tank and seemed to thrive very well. They multiplied enormously and schools of them could be seen everywhere along the tank edges. Mosquito larvae were rarely found. It was intended to use this tank as a nursery for *Gambusia* fish, and for several months they did very well in it. Thousands were removed for stocking wells in the neighbourhood. But, for some reason, the *Gambusia* disappeared before the 1941 malaria season started. The only plausible explanation seems to be that predatory fish established themselves in the tank, in spite of our precautions, and destroyed the *Gambusia*.

### IRRIGATION CANALS.

The edges of irrigation canals were important breeding places of *A. culicifacies*, and in this area, especially near Senjayakollai hamlet, these canals were particularly bad, because their bunds were frequently breached. Water flowed through these breaches and created vast additional 'fallow fields'. The problem of breached bunds was solved by installing proper culverts and that of canal breeding by installing and operating automatic paris green distributors.

#### (a) CULVERTS.

A branch canal went around two sides of Senjayakollai hamlet, between the town and the main Pattukkottai-Tanjore road, which was the only means of approach to market for the villagers. The canal passed the village at a higher elevation than the village site, crossing the village road which led to the main highway. Yet, no culverts were provided for this village road when the branch canal was built. Thus high bunds isolated the village from the highway. Quite naturally, the villagers had to get out and so they drove their carts across the



canal bunds, partially destroying them. Consequently, when irrigation water was turned on each year, it flowed through breached bunds, down cart tracks and into the village. Much of the hamlet site was one huge 'fallow field', with *A. culicifacies* breeding in great numbers.

This condition in Senjayakollai was typical of inadequate planning of channel layouts which was seen in many villages in Pattukkottai taluk. Much valuable water has gone to waste and many malaria as well as other hazards have thus been created. So one of the first items in the project was the installation of two culverts at advantageous points on cart tracks leading into the village. Bathing and dhobi ghats were included as a part of the culvert structure.

These two culverts made it possible to confine all normal water to the canal, so that much of the hamlet site became dry. Carts and pedestrians could go to market or to fields without crossing through canal bed and breaching bunds. Bathing and washing were simplified. Irrigation water was saved. In fact, it was estimated that the water saved would pay for the culverts within two irrigation seasons.

#### (b) AUTOMATIC PARIS GREEN DISTRIBUTORS.

An attempt was made to control larval breeding in branch channels by fluctuation of water level and flushing. This failed as explained below :—

During the 1938 season, some paris green distribution by the dustless method mentioned above was carried out successfully. But, since it was desirable to make all control measures as nearly automatic as possible, the use of automatic paris green distributors was tried (Russell and Eaton, 1934). A mixture of 2 per cent paris green diluted with finely ground charcoal was used in the distributors.

These distributors functioned reasonably well during the 1939 season. Inherent weaknesses which developed were strengthened, and an improved type of distributor was used successfully in 1940 and 1941 (Russell and Knipe, 1942). The distributors were operated twice weekly for about 8 hours, each machine effectively controlling at least 1,500 feet of canal (3,000 lineal feet of margin). It was remarkable how the paris green particles were taken by surface water and wind currents into all small marginal pools along canal banks.

As the result of our experiments we do not recommend the use of this machine on canals where the velocity of water exceeds approximately 1.6 feet per second. Greater velocities interfere with distribution of paris green toward canal edges. But for canals of lesser velocities the machines were satisfactory.

#### (c) FLUCTUATION FAILURE.

There has been considerable speculation regarding the effect of rapid fluctuation of water level on mosquito larvæ in canals. Therefore, fluctuation was tried out in branch canals, but without success. The canal gradient was not sufficient to draw down the water fast enough to strand larvæ. Nor was the gradient



sufficient to permit flushing out of larvæ when water was re-introduced. Consequently, attempts at stranding and flushing in canals ended in failure.

One fact, however, seemed to be established. The top of the sluice-gate through which the water passed from the main canal into branch canals was submerged six inches below the water surface in the main canal. *A. culicifacies* larvæ apparently never passed alive through that submerged sluice. Over a period of several weeks all the water remaining in the branch canal, after the head sluice was shut for the intermittent irrigation period, was very thoroughly treated with paris green. This was done for a distance of 2,000 feet below the sluice. A careful search was made for larvæ before the head sluice was again opened. After the head sluice was opened, daily searches were made for the aquatic stages. They were found only as they might be expected to develop through the stages from egg to pupæ. In other words, no second, third, or fourth stage larvæ, or pupæ were found for several days after the head sluice was opened. Repeated trials, always with the same result, seemed to indicate that *A. culicifacies* larvæ do not usually drift through an orifice, such as a sluice-gate, if that gate is submerged six inches or more below water surface of the feeder canal.

#### WET FALLOW FIELDS.

According to our own definition, wet fallow fields may be divided into two types as follows :—

1. Fields under preparation for rice planting, or having rice not yet a foot or more high ;
2. Unplanted fields and waste lands which during the irrigation season may lie more or less permanently under water, varying in depth from a fraction of an inch to several inches, and usually supporting some natural vegetation.

Fields planted with rice, but on which rice plants were still less than 12 inches high, were, of course, not true fallow fields, but in respect of *A. culicifacies* they were exactly like fallow fields.

The field which was being prepared for cultivation was usually not dangerous from the point of view of mosquito production, because the water was frequently agitated, muddy and polluted by manure to some extent. *A. culicifacies* did not choose it as a common breeding place. But there were considerable numbers of *A. culicifacies* larvæ in growing ricefields before the rice was a foot high. After the rice was a foot or more high, *A. culicifacies* was no longer found.

By far the greater number of *A. culicifacies* came from true fallow fields, unplanted fields and waste land, under a shallow depth of water. These were the fields which had to be dried periodically throughout the irrigation season if *A. culicifacies* was to be controlled.



The most logical method of dealing with the fallow fields included in the above definition seemed to be intermittent irrigation, and it was used successfully as described in the next section.

### INTERMITTENT IRRIGATION.

Soon after we began to deal with 'untidy water' in the area, it began to appear that 'minor engineering', such as borrowpit filling, putting field channels in order, and sanitating wells and tanks, might be insufficient as a malaria control measure. Too much water remained on fallow fields, undoubtedly accounting for a major percentage of the vector mosquito population. Therefore, to control mosquito breeding in these fields, intermittent irrigation was introduced.

This early supposition that minor engineering alone would not have sufficient effect on malaria, has not been entirely substantiated. The 1941 malaria spleen and parasite rates for Enadi, a hamlet within the seven-mile zone, indicate a notable reduction in malaria (Table I). Yet Enadi has not been within the section subjected to intermittent irrigation, but has had only control by minor engineering.

Intermittent irrigation, or as it is locally called the 'turn system', is a term used in the application of water to land. When the flow of irrigation water to land is more or less regularly interrupted, that land is said to be intermittently irrigated. During the period when water is interrupted in its flow, the land is undergoing a process of drying. Drying may take place rapidly or slowly, depending upon several factors, including soil structure, soil texture, rate of evaporation and level of subsoil water table.

Experiments conducted since 1938 have proved that rice yield and quality are not affected by a properly controlled system of intermittent irrigation (Russell *et al.*, 1942). These experiments indicated that mosquito larvæ were invariably destroyed when the *surface film of water on the soil disappeared*. Local soil types were found to dry to the point where the surface film disappeared, in approximately  $2\frac{1}{2}$  days. Therefore,  $2\frac{1}{2}$  days was selected as the 'drying period' in the scheme of intermittent irrigation formulated for this area.

Before intermittent irrigation could be effectively established as an engineering practice in the control of *A. culicifacies*, it was necessary to have absolute control of the flow of water to various areas and for specified times. This meant control in branch feeder channels and in all field channels. A watertight Calco gate was therefore installed at the branch canal head sluice, and other watertight Calco gates at every field channel sluice. The gates were locked in position (open or closed) so that they could not be tampered with. In all, nine gates were installed in the area. This gave complete control of all irrigation water entering the area.

Since control over the flow of water was complete, it was hoped that control of mosquito breeding could be accomplished in the branch channel, in all field



channels and in all fallow fields. Success was achieved to a remarkable degree in field channels and in fallow fields, but not in the branch canal.

Development of *A. culicifacies* larvæ in field channels was effectively stopped by intermittent irrigation. The drying period was quite sufficient to dry all bed pools in these channels, with the result that properly constructed and maintained field channels ceased to be a source of breeding of *A. culicifacies* under intermittent irrigation. Breeding was prevented by the complete drying of the channel beds, which took place usually within 36 hours after water flow was stopped.

The drying period (time taken for disappearance of surface film of water) which seemed to be effective for the control of larvæ on the soil type of this area, was between two and three days. Since the period of development of the mosquito larvæ was from seven to nine days, it appeared that a programme for intermittent irrigation could be established on a weekly routine basis of  $4\frac{1}{2}$  days of irrigation and  $2\frac{1}{2}$  days of drying. This practice was followed during the irrigation seasons of 1940 and 1941. The results obtained were very satisfactory. Breeding was stopped on practically all true fallow fields and was inhibited in newly planted ricefields to the point where it was of very little importance.

It was quite natural that the ryots should offer objections to the practice when it was inaugurated. But no field or rice was allowed to suffer for lack of water. Once the ryots appreciated that their interests were being safeguarded, practically all of them willingly accepted the practice. The rice crop in the area in 1940 was as good as in any adjoining district. This fact was attested by competent authorities, both interested and disinterested parties. The same system of intermittent irrigation was again followed during the 1941 season. Crops were entirely satisfactory and there was very little complaining by ryots.

### COSTS.

The malaria control programme described above required an expenditure of money, as does all public health work, but it was not so great as one might estimate. The following list summarizes the cost figures :—

			Rs.	as.	p.
Total cost (7 square miles)	..	..	14,841	4	11*
Cost per square mile (approx.)	..	..	2,100	0	0
Cost per acre (total acreage)	..	..	3	4	0
Cost per irrigated acre	..	..	7	13	0
Cost <i>per capita</i>	..	..	4	6	0

It is believed that Rs. 4-6-0 *per capita* is not a high price to pay for reasonably permanent malaria control measures, especially in rural areas. Maintenance of

---

\* A rupee consists of 16 annas, each anna of 12 pies. At the present rate of exchange a rupee is worth £0-1-6 or U. S. \$0.30.



## 628 *Control of Rural Irrigation Malaria by Antilarval Measures.*

course, remains a problem. But we completed one full year of maintenance operations—from 1.xi.40 to 31.x.41—at a remarkably low cost. Accurate records for this period reveal the following complete expenditure :—

					Rs.	as.	p.
Labour	..	..	..	..	425	0	0
Supervision	...	..	..	..	125	0	0
Total Rs.					550	0	0
Maintenance per square mile of area (approx.)	..				78	9	0
Maintenance per acre (total acreage)	..				0	2	0
Maintenance per irrigated acre	..				0	4	10
Maintenance <i>per capita</i>	..	..	..	..	0	1	11

The question may arise at this point as to the value of malaria control in Pattukkottai taluk. It is not always easy to answer this question. The facts are that, as shown below, a notable reduction in malaria has been accomplished. This reduction has cost approximately Rs. 4-6-0 *per capita*. Maintenance of the system for the first year after completion of the job has cost Re. 0-1-11 *per capita* (approximately U. S. \$0.03 $\frac{1}{4}$  or £0-0-2 $\frac{1}{4}$ ). Considering the fact that malarial-economic surveys in this area show that malaria itself costs the ryots Rs. 2-8-0 *per capita* per year, not counting lost wages, malaria control by antilarval measures would seem to be a reasonable proposition (Russell and Menon, 1942).

### RESULTS.

The work performed in this project is summarized in Table III, first by years, and then by totals.

The main objective of these four years of activities has been the control of malaria. Control measures were introduced slowly, step by step, and with no attempt to gain spectacular results. Rather, an effort was made to introduce measures which would not only influence malaria control, but would also tend to raise the standard of living of the community. Better agriculture, better roads, better bathing and washing facilities, as well as better health, would all help toward raising that standard. While all these points were entirely secondary in the project, yet their importance was never forgotten.

Spleen and parasite rates are shown in Tables I and II. They indicate that the control measures did in fact greatly reduce the amount of malaria transmission. For example, the spleen rate in Senjayakollai hamlet was reduced from 48 per cent in 1937 to 4 per cent in 1941, and the parasite rate from 42 per cent in 1937 to *nil* in the 1941 malaria season. In the similar contrast village of Pappanadu, the spleen rate in 1937 was 53 and it was 57 in 1941. Parasite rates in this uncontrolled village were 43 per cent in 1937 and 48 per cent in 1941.



## SUMMARY.

An experiment is reported on the control of irrigation malaria in an area of 7 square miles in South India, by antilarval measures, including some 55,000 cubic yards of filling, draining, use of *Gambusia*, of chopped cactus, of paris green and of intermittent irrigation. The programme extended over a period of four years, affected a population of some 3,390 persons in a strictly rural area, and cost Rs. 4-6-0 (\$1.31 or £0-6-6) *per capita*. Maintenance costs during one year of observation after completion of the engineering measures were only Re. 0-1-11 *per capita* (\$0.03½ or £0-0-2½).

The control measures resulted in a notable fall in spleen and parasite rates.

## REFERENCES.

- BARBER, M. A., RICE, J. B., and MANDEKOS, A. (1936). A dustless method of diluting and spreading paris green in malaria control. *Amer. J. Hyg.*, **24**, pp. 41-44.
- HACKETT, L. W., RUSSELL, P. F., SCHARFF, J. W., and SENIOR WHITE, R. (1938). The present use of naturalistic measures in the control of malaria. *Bull. Health Org. League of Nations*, **7**, 6, pp. 1016-1064.
- RUSSELL, P. F. (1938) .. Malaria due to defective and untidy irrigation. A preliminary discussion. *J. Mal. Inst. Ind.*, **1**, 4, pp. 339-349.
- Idem* (1941) .. Naturalistic methods of malaria control. Symposium on human malaria. Pub. No. 15, Am. Assoc. for Advance Science, Washington.
- RUSSELL, P. F., and EATON, L. S. (1934) An automatic distributing machine for paris green mixtures. *Philippine J. Sci.*, **53**, 4, pp. 497-503.
- RUSSELL, P. F., and KNIPE, F. W. (1939) Malaria control by spray-killing adult mosquitoes. First season's results. *J. Mal. Inst. Ind.*, **2**, 3, pp. 229-237.
- Idem* (1940) .. Malaria control by spray-killing adult mosquitoes. Second season's results. *Ibid.*, **3**, 4, pp. 531-541.
- Idem* (1941) .. Malaria control by spray-killing adult mosquitoes. Third season's results. *Ibid.*, **4**, 2, pp. 181-197.
- Idem* (1942) .. A machine for automatic distribution of paris green mixtures. *Amer. J. Trop. Med.* (In press).
- RUSSELL, P. F., KNIPE, F. W., and RAO, H. R. (1942). On the intermittent irrigation of ricefields to control malaria in South India. *J. Mal. Inst. Ind.*, **4**, 3, pp. 321-340.
- RUSSELL, P. F., KNIPE, F. W., and RAO, T. R. (1940). On using water instead of dust for diluting paris green in malaria control. *Ind. Med. Gaz.*, **75**, 12, pp. 740-742.
- RUSSELL, P. F., and MENON, M. K. (1942) A malarial-economic survey in rural South India. *Ind. Med. Gaz.*, **77**, 3, pp. 167-180.
- RUSSELL, P. F., MENON, M. K., and RAO, T. R. (1938). Epidemiology of malaria in Pattukkottai Taluk, Tanjore District, Madras Presidency, India. *J. Mal. Inst. Ind.*, **1**, 3, pp. 285-326.
- RUSSELL, P. F., and RAO, H. R. (1940) The Anopheles of ricefields in south-eastern Madras. *Ibid.*, **3**, 4, pp. 427-446.
- RUSSELL, P. F., and RAO, T. R. (1940a) On habitat and association of species of anopheline larvae in south-eastern Madras. *Ibid.*, **3**, 1, pp. 153-178.
- Idem* (1940b) ... Natural malaria infections in some South Indian anophelines, with special reference to *A. culicifacies*. *Ibid.*, **3**, 4, pp. 543-562.
- Idem* (1941) .. On seasonal prevalence of Anopheles species in south-eastern Madras. *Ibid.*, **4**, 2, pp. 263-296.



630 *Control of Rural Irrigation Malaria by Antilarval Measures.*

TABLE I.

*Spleen rates in Senjayakollai project area and contrast village of Pappanadu, 1937 to 1941.*

Village.		YEAR AND SEASON.									
		1937.		1938.		1939.		1940.		1941.	
		Off	Mal.	Off	Mal.	Off	Mal.	Off	Mal.	Off	Mal.
<i>Experimental :</i>											
Senjayakollai ..	..	..	48	33	44	12	26	13	16	7	4
Enadi ..	..	43	54	38	59	44	53	38	42	21	19
Aladikumalai ..	..	35	56	41	57	35	55	35	37	18	11
<i>Contrast :</i>											
Pappanadu ..	..	40	53	39	55	37	46	40	53	40	57

*Note.*—Mal. = malaria season.

TABLE II.

*Parasite rates in Senjayakollai project area and contrast village of Pappanadu, 1937 to 1941.*

Village.		YEAR AND SEASON.									
		1937.		1938.		1939.		1940.		1941.	
		Off	Mal.	Off	Mal.	Off	Mal.	Off	Mal.	Off	Mal.
<i>Experimental :</i>											
Senjayakollai ..	..	..	42	28	31	33	32	21	15	6	0
Enadi ..	..	37	50	24	37	32	41	22	34	14	10
Aladikumalai ..	..	33	49	32	44	18	46	20	20	12	3
<i>Contrast :</i>											
Pappanadu ..	..	19	43	17	22	20	38	25	47	28	48

*Note.*—Mal. = malaria season.



TABLE III.

*Work accomplished in Senjayakollai project area by years.*

Particulars.	1938.	1939.	1940.	1941.	TOTAL.
Lineal feet of canals excavated, realigned and constructed ..	17,724	23,246	18,165	7,472	66,607
Cubic yards of earth dug from canals .. ..	1,672	2,349	4,546	242	8,809
Cubic yards of fill ..	3,811	32,304	18,946	249	55,310
Culverts constructed ..	2	13	Nil	Nil	15
Land reclaimed for rice culture by filling, tank filling, field lowering, in acres ..	..	..	..	..	35.5
Wells stocked with <i>Gambusia</i> ..	Nil	Nil	779	685	1,464
Calco gates installed ..	Nil	Nil	9	Nil	9
Man days labour .. ..	1,662	10,641	8,682	744	21,729
	Rs. a. p.	Rs. a. p.	Rs. a. p.	Rs. a. p.	Rs. a. p.
Labour cost .. ..	580 9 0	4,031 10 0	3,435 1 3	492 12 7	8,540 0 10
Supervision .. ..	126 0 0	689 3 7	1,566 12 5	338 8 0	2,720 8 0
Wages (other than labour) ..	Nil	35 7 9	175 10 6	200 11 6	411 13 9
Transport .. ..	63 1 0	150 9 0	146 14 9	17 15 6	378 8 3
Material .. ..	287 12 10	1,424 5 5	794 6 7	283 13 3	2,790 6 1
TOTAL ..	1,057 6 10	6,331 3 9	6,118 13 6	1,333 12 10	14,841 4 11







## ABSTRACT.

### OBSERVATIONS ON THE RELATIVE UTILITY OF *GAMBUSIA AFFINIS* AND *PANCHAX PARVUS* IN THE CONTROL OF MOSQUITO BREEDING IN WELLS AND TANKS.\*

BY

R. BHASKER RAO, M.B., B.S., B.SC.

(Health Officer),

AND

H. RAMOO, B.A.

(Entomological Assistant)

(Antimalarial Scheme, Pattukkottai, Madras Presidency).

[September 10, 1942.]

A SERIES of experiments was carried out at Pattukkottai, Tanjore District, Madras Presidency, to test the relative efficacy of *Gambusia affinis*, an American fish which has been acclimatized in India for the last 12 years, and of *Panchax parvus*, a local indigenous species, in the control of mosquito breeding. As the result of observations made both in the laboratory and in the field, the following conclusions were reached :—

1. *Gambusia affinis* is more adaptable to diverse environmental conditions than *Panchax parvus*, which thrives best in natural surroundings and is not suited for use in confined waters.

2. *Gambusia affinis* thrives better than *Panchax parvus* in wells and is more effective for controlling mosquito breeding in them.

---

\* A copy of the original manuscript has been placed in the Library of the Malaria Institute of India, Kasauli. This is available on loan to workers who wish to consult it. (Editor.)



634 *The Relative Utility of Gambusia affinis and Panchax parvus.*

3. *Gambusia affinis* is easier to rear in large numbers in artificial hatcheries than *Panchax parvus*.

4. *Gambusia affinis* devours more larvæ and at a quicker rate than *Panchax parvus*.

The authors consider that *Gambusia affinis* has proved of great utility in the control of mosquito breeding in Pattukkottai, both in wells and in tanks.

G. C.



STATUS OF *ANOPHELES CULICIFACIES* AS A  
VECTOR OF MALARIA IN THE EASTERN  
SATPURA RANGES.

EDITORIAL NOTE.

IN an article entitled ' On the importance of *Anopheles pallidus* as a carrier of malaria in Udaipur State, Central Provinces ' by D. N. Roy and T. C. Biswas, which appeared in the *Journal of the Malaria Institute of India*, Vol. IV, No. 3, June 1942, it was erroneously stated that *A. culicifacies* had been reported to be the vector of malaria in the Eastern Satpuras by Senior White and Adhikari (1940).

The conclusions arrived at by Senior White and Adhikari, as set out in the summary of their paper, were :—

- (i) In the Eastern Satpuras the principal vectors are *A. fluviatilis* and *A. varuna*.
- (ii) In this area, *A. culicifacies* plays at most a very small part in malaria causation.







## INDEX OF AUTHORS.

PAGE

### B

BISWAS, T. C., *see* ROY and BISWAS.

### C

- CHOPRA, R. N., ROY, D. N., and GHOSH, S. M. The Insecticidal and Larvicidal Action of the Essential Oils of *Ocimum basilicum* (Linn.) and *Ocimum sanctum* (Linn.) .. .. . 109
- CHOUDHURY, M. A., *see* CLARK and CHOUDHURY.
- CLARK, R. H. P., and CHOUDHURY, M. A. Observations on *Anopheles leucosphyrus* in the Digboi Area, Upper Assam .. .. . 103
- COVELL, G. Antimalaria Operations in Delhi. Part III .. .. . 1
- COVELL, G., and PRITAM SINGH. Malaria in the Coastal Belt of Orissa .. 457

### D

- DAS, S., *see* VISWANATHAN, DAS and OOMMEN.
- DE BURCA, B., and MOHD. YUSAF. A New Variation of *Anopheles gambiae* 447
- DIKSHIT, B. B. Malaria Immunity in the Rhesus Monkey .. .. . 199

### G

GHOSH, S. M., *see* CHOPRA, ROY and GHOSH.

### I

- IYENGAR, M. O. T. Studies on Malaria in the Deltaic Region of Bengal .. 435
- IYER, P. V. S., *see* SHORTT, MENON and IYER.
- IYER, P. V. S., SHORTT, H. E., and MENON, K. P. The Stage of *Plasmodium gallinaceum* found in the Incubation Period—Second Observation . 179

### J

- JACOB, V. P., *see* RUSSELL and JACOB.
- JAMES, E. R. Abstract. The Investigation of the High Incidence of Malaria in Mawchi Mines Area, situated in Karenni States, Burma, and the Measures adopted to Combat and Control the Disease .. .. . 153



	PAGE
JOB, T. J. On the Comparative Efficacy and Relative Costs of Biological and Chemical Methods of Mosquito Control in Clean-weeded Railway Borrowpits at Fuleshwar, Bengal .. .. .	211

## K

KHAN, B. M. Abstract. Malaria in the Tista Valley, Darjeeling District ..	421
KNIFE, F. W., <i>see</i> RUSSELL and KNIFE.	
KNIFE, F. W., <i>see</i> RUSSELL, KNIFE and RAO.	
KNIFE, F. W., and RUSSELL, P. F. A Demonstration Project in the Control of Rural Irrigation Malaria by Antilarval Measures .. .. .	615

## M

MENON, K. P., <i>see</i> IYER, SHORTT and MENON.	
MENON, K. P., <i>see</i> SHORTT, MENON and IYER.	
MENON, M. A. U. On the Abdominal Tergites of Adult <i>Mansonioides</i> Theobald, 1907. (Diptera, Culicidæ) .. .. .	207
MOHAN, BADRI NATH, <i>see</i> MULLIGAN, RUSSELL and MOHAN.	
MOHAN, BADRI NATH, <i>see</i> RUSSELL, MULLIGAN and MOHAN.	
MOHD. YUSAF, <i>see</i> DE BURCA and MOHD. YUSAF.	
MULLIGAN, H. W., <i>see</i> RUSSELL, MULLIGAN and MOHAN.	
MULLIGAN, H. W., RUSSELL, P. F., and MOHAN, BADRI NATH. Active Immunization of Fowls against <i>Plasmodium gallinaceum</i> by Injections of Killed Homologous Sporozoites .. .. .	25

## O

OOMMEN, A. V., <i>see</i> VISWANATHAN, DAS and OOMMEN.	
--	--

## P

PANIGRAHI, R. G. Abstract. Malaria in Puri .. .. .	423
PRITAM SINGH, <i>see</i> COVELL and PRITAM SINGH.	

## R

RAMOO, H., <i>see</i> RAO and RAMOO.	
RAO, G. R. Abstract. Malaria in the Jharia Mining Settlement, Bihar ..	307
RAO, H. R., <i>see</i> RUSSELL, KNIFE and RAO.	
RAO, P. J., <i>see</i> RAO, ROY and RAO.	
RAO, R. B., and RAMOO, H. Some Notes on the Practical Aspects of Mosquito Control in Wells and Tanks by the Use of Larvivorous Fish ..	341



	PAGE
RAO, R. B., and RAMOO, H. The Control of Mosquito-Breeding in Canal Distributaries by growing Certain Plants on their Banks .. ..	409
RAO, R. B., and RAMOO, H. Abstract. Observations on the Relative Utility of <i>Gambusia affinis</i> and <i>Panchax parvus</i> in the Control of Mosquito Breeding in Wells and Tanks .. .. .	633
RAO, T. R., see RUSSELL and RAO.	
RAO, V. V., ROY, B. B., and RAO, P. J. Observations on the Swarming and Pairing of <i>A. sundanicus</i> (Rodenwaldt) and <i>A. subpictus</i> (Grassi) ..	405
ROY, B. B., see RAO, ROY and RAO.	
ROY, D. N., and BISWAS, T. C. On the Importance of <i>Anopheles pallidus</i> as a Carrier of Malaria in Udaipur State. Central Provinces ..	417
ROY, D. N., see CHOPRA, ROY and GHOSH.	
RUSSELL, P. F., see KNIPE and RUSSELL.	
RUSSELL, P. F., see MULLIGAN, RUSSELL and MOHAN.	
RUSSELL, P. F., and JACOB, V. P. On the Epidemiology of Malaria in the Nilgiris District, Madras Presidency .. .. .	349
RUSSELL, P. F., and KNIPE, F. W. Malaria Control by Spray-killing Adult Mosquitoes. Third Season's Results .. .. .	181
RUSSELL, P. F., KNIPE, F. W., and RAO, H. R. On the Intermittent Irrigation of Ricefields to Control Malaria in South India .. ..	321
RUSSELL, P. F., and RAO, T. R. On Seasonal Prevalence of <i>Anopheles</i> Species in South-Eastern Madras .. .. .	263
RUSSELL, P. F., MULLIGAN, H. W., and MOHAN, BADRI NATH. Specific Agglutinogenic Properties of Inactivated Sporozoites of <i>P. gallinaceum</i> ..	15
RUSSELL, P. F., MULLIGAN, H. W., and MOHAN, BADRI NATH. Active Immunization of Fowls against Sporozoites but not Trophozoites of <i>Plasmodium gallinaceum</i> by Injections of Homologous Sporozoites ..	311
RUSTOMJEE, K. J., see SIVALINGAM and RUSTOMJEE.	

## S

SALITERNIK, Z., and THEODOR, O. On a New Variety of <i>Anopheles turkhudi</i> from Palestine .. .. .	429
SEN, P. Aquatic Plants in the Ecology of Anopheline Mosquitoes ..	113
SEN, P. On the Microsporidia infesting some Anophelines of India ..	257
SENIOR WHITE, R. Observations on the Adult Habits of <i>Anopheles fluviatilis</i> and <i>Anopheles varuna</i> .. .. .	57
SHORTT, H. E., see IYER, SHORTT and MENON.	



	Page
SHORTT, H. E., MENON, K. P., and IYER, P. V. S. The Natural Host of <i>Plasmodium gallinaceum</i> (Brumpt, 1935) .. .. .	175
SIVALINGAM, V., and RUSTOMJEE, K. J. Spleen and Parasite Surveys in Ceylon .. .. .	155

## T

THEODOR, O., <i>see</i> SALITERNIK and THEODOR.	
THOMSON, R. C. M. Studies on the Behaviour of <i>Anopheles minimus</i> . Part IV. The Composition of the Water and the Influence of Organic Pollution and Silt .. .. .	63
THOMSON, R. C. M. Studies on the Behaviour of <i>Anopheles minimus</i> . Part V. The Behaviour of Adults in Relation to Feeding and Resting in Houses .. .. .	217
THOMSON, R. C. M. Studies on the Behaviour of <i>Anopheles minimus</i> . Part VI. Observations on the Cold Weather Breeding Places .. .. .	247
THOMSON, R. C. M. Studies on the Behaviour of <i>Anopheles minimus</i> . Part VII. Further Studies on the Composition of the Water in Breeding Places and the Influence of Organic Pollution .. .. .	595
THOMSON, R. C. M. Studies on the Behaviour of <i>Anopheles minimus</i> . Part VIII. The Naturalistic Control of <i>A. minimus</i> in Shallow Earth Wells .. .. .	611

## V

VISWANATHAN, D. K. Experimental Malaria Control in a Hyperendemic Tea Garden in Upper Assam by the Use of Pyroicide 20 as an Insecticidal Spray .. .. .	35
VISWANATHAN, D. K. The Utility of Malaria Parasite Indices in Infants in the Study of Malaria .. .. .	139
VISWANATHAN, D. K. Malaria Control by Spray-killing Adult Anophelines, Second Season's Results: With Special Reference to the Effects of this Measure on the Longevity and Infectivity of <i>Anopheles minimus</i> .. .. .	393
VISWANATHAN, D. K., DAS, S., and OOMMEN, A. V. Malaria-carrying Anophelines in Assam, with Special Reference to the Results of Twelve Months' Dissections .. .. .	297

## W

WIJESUNDARA, D. P. Notes on the Mosquito Fauna of Rot-Holes in Trees and Bamboo Stumps in Ceylon .. .. .	451
--	-----



## INDEX OF SUBJECTS.

- ANOPHELES**, aquatic plants in the ecology of, 113; in Assam, malaria-carrying, 297; Microsporidia infesting, 257; prevalent in Madras, 263; *culicifacies* as malaria vector in Satpura Ranges, status of, 635; *fluviatilis*, adult habits of, 57; *gambiae*, a new variation of, 447; *leucosphyrus*, observations on, 103; *minimus*, behaviour of, 63, 217, 247, 595, 611, effect of spraying on longevity and infectivity of, 393, naturalistic control of, 611; *pallidus*, as a carrier in Udaipur State, 417; *subpictus*, swarming and pairing of, 405; *sundaicus*, swarming and pairing of, 405; *turkhudi*, a new variety of, 429; *varuna*, adult habits of, 57.
- ASSAM**, control by spray-killing in, 35; malaria-carrying anophelines in, 297; observations on *A. leucosphyrus* in, 103.
- BENGAL**, malaria in Darjeeling District in, 421; malaria in deltaic, 435; mosquito control in railway borrowpits in, 211.
- BIHAR**, malaria in Jharia Mining Settlement in, 307.
- BURMA**, incidence of malaria in, 153.
- CEYLON**, mosquito fauna of rot-holes in trees and bamboo stumps in, 451; spleen and parasite surveys in, 155.
- CENTRAL PROVINCES**, importance of *A. pallidus* as a carrier in Udaipur State in, 417, 635.
- CONTROL** by antilarval measures, 615; by fish in wells and tanks, 341, 633; by intermittent irrigation, 321; by spray-killing of adult mosquitoes, 35, 181, 393; efficacy and costs of biological and chemical methods of, 211; in Mawchi Mines area in Burma, malaria, 153; of *A. minimus* in shallow earth wells, naturalistic, 611; of breeding in canal distributaries by growing plants, 409.
- CULICIFACIES**, see Anopheles.
- DELHI**, antimalaria operations in, 1.
- FISH**, control in wells and tanks by, 341, 633.
- FLUVIATILIS**, see Anopheles.
- GALLINACEUM**, see Plasmodium.
- GAMBIAE**, see Anopheles.
- IMMUNITY** in Rhesus monkey, malaria, 199.
- IMMUNIZATION** of fowls against *P. gallinaceum*, active, 25, 311.
- IRRIGATION** of ricefields to control malaria, intermittent, 321; malaria, antilarval control of, 615.
- LEUCOSPHYRUS**, see Anopheles.
- MADRAS**, epidemiology of malaria in, 349; seasonal prevalence of Anopheles in, 263.
- MALARIA**, *A. pallidus* as a carrier of, 417; control in Assam, 35; control in South India, 321; epidemiology of, 349; immunity in, 199; in coastal belt of Orissa, 457; in Darjeeling District, 421; in deltaic Bengal, 435; in Jharia, 307; in Mawchi Mines area, 153; in Puri, 423; in Satpura Ranges, 635; in Udaipur State, 417, 635; parasite indices, utility of, 139.
- MANSONIOIDES**, abdominal tergites of, 207.
- MICROSPORIDIA** infesting Anopheles, 257.
- MINIMUS**, see Anopheles.
- NATURALISTIC CONTROL**, see Control.
- OCIMUM BASILICUM**, see Plants.
- OCIMUM SANCTUM**, see Plants.
- ORISSA**, malaria in, 423, 457.
- PALESTINE**, a new variety of *A. turkhudi* from, 429.
- PALLIDUS**, see Anopheles.
- PLANTS**, control of breeding by growing, 409; insecticidal and larvicidal action of oils of *Ocimum basilicum* and *Ocimum sanctum*, 109; in the ecology of anopheline mosquitoes, aquatic, 113.
- PLASMODIUM gallinaceum**, active immunization of fowls against, 25, 311; found in incubation period, stage of, 179; natural host of, 175; specific agglutinogenic properties of inactivated sporozoites of, 15.



RAILWAY borrowpits, control of breeding in, 211.

SOUTH INDIA, control by intermittent irrigation in, 321.

SPOROZOITES, active immunization of fowls by injections of homologous, 25, 311; of *P. gallinaceum*, specific agglutinogenic properties of inactivated, 15.

SPRAY-KILLING of adult mosquitoes, 35, 181, 393.

*SUBPICTUS*, *see* Anopheles.

*SUNDAICUS*, *see* Anopheles.

*TURKHUDI*, *see* Anopheles.

*VARUNA*, *see* Anopheles.











**I. A. R. I. 75.**

IMPERIAL AGRICULTURAL RESEARCH  
INSTITUTE LIBRARY  
NEW DELHI.

[illegible]